Evaluation of Ketanserin in the Prevention of Restenosis After Percutaneous Transluminal Coronary Angioplasty

A Multicenter Randomized Double-Blind Placebo-Controlled Trial

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Background. **Ketanserin is a serotonin S_2-receptor antagonist that inhibits the platelet activation and vasoconstriction induced by serotonin and also inhibits the mitogenic effect of serotonin on vascular smooth muscle cells.**

Methods and Results. **We conducted a randomized, double blind, placebo-controlled trial to assess the effect of ketanserin in restenosis prevention after percutaneous transluminal coronary angioplasty (PTCA). Patients received either ketanserin (loading dose, 40 mg 1 hour before PTCA; maintenance dose, 40 mg bid for 6 months) or matched placebo. In addition, all patients received aspirin for 6 months. Coronary angiograms before PTCA, after PTCA, and at 6 months were quantitatively analyzed. Six hundred fifty-eight patients were entered into the intention-to-treat analysis. The primary clinical end point of the study was the occurrence between PTCA and 6 months of any one of the following: cardiac death, myocardial infarction, the need for repeat angioplasty, or bypass surgery. It also included the need for revascularization actuated by findings at 6-month follow-up angiography. The primary clinical end point was reached by 92 (28%) patients in the ketanserin group and 104 (32%) in the placebo group (RR, 0.89; 95% CI, 0.70, 1.13; $P=.38$). Quantitative angiography after PTCA and at follow-up was available in 592 patients (ketanserin, 287; control, 305). The mean difference in minimal lumen diameter between post-PTCA and follow-up angiogram (primary angiographic end point) was 0.27±0.49 mm in the ketanserin group and 0.24±0.52 mm in the control group (difference, 0.03 mm; 95% CI, −0.05, 0.11; $P=.50$).**

Conclusions. **Ketanserin at the dose administered in this trial failed to reduce the loss in minimal lumen diameter during follow-up after PTCA and did not significantly improve the clinical outcome. (*Circulation.* 1993;88[part 1]:1588-1601.)**

KEY WORDS • **ketanserin** • **restenosis** • **angioplasty**

Percutaneous transluminal coronary angioplasty (PTCA) is increasingly used to alleviate coronary artery stenosis and its ischemic repercussions. Despite an upgrading of technology, the technique remains limited by restenosis of the dilated segment in 25% to 35% of cases; this occurs mainly within 4 to 6 months after PTCA.[1] In the early phase after PTCA, restenosis is mainly due to thrombosis.[2,3] Later on, vascular smooth muscle cell proliferation and synthetic activity result in pronounced intimal thickening and play a major role in renarrowing the lumen of the dilated vessels.[4-7] These phenomena leading to restenosis are initiated by endothelial denudation and intimal and medial damage. This first gives rise to adhesion and subsequently to accumulation of activated platelets on the vascular lesion,[4,8-10] which results in the release of various platelet-derived products, including platelet-derived growth factor (PDGF), 5-hydroxytryptamine (5-HT), prostaglandin endoperoxides, thromboxane A_2, and adenosine diphosphate (ADP).[11-14] Platelet-derived prostanoids, ADP, 5-HT, and thrombin are primarily involved in the platelet-dependent genesis of occluding arterial thrombi and of vasospasms in areas with deficient endothelium-dependent relaxation,[11,15-18] while PDGF in particular as a potent mitogen for vascular smooth muscle cells in vitro[19,20] has been implicated in intimal hyperplasia in reaction to vessel wall damage.[20,21] However, platelet-derived 5-HT also stimulates migration and proliferation of vascular smooth muscle cells[19,22-25] and promotes the synthesis of collagen and proteoglycans by isolated cells.[24,26,27] The 5-HT-induced stimulation of platelet aggregation as well as stimulation of vascular smooth muscle cell proliferation appear to be mediated by the 5-HT_2

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serotonin receptor subtype, since these effects were blocked by the selective 5-HT_2 antagonist ketanserin. In a small placebo-controlled study, Klein et al[28] found that intravenous infusion with ketanserin added to acetyl salicylic acid, heparin, and a calcium antagonist appeared to reduce the incidence of occlusion or early reduction of diameter 24 hours after angioplasty.

The present study, referred to as the Post-Angioplasty Restenosis Ketanserin (PARK) Study, was carried out to further evaluate the role of ketanserin in the prevention of restenosis and its clinical complications after PTCA.

Methods

Study Population

Consecutive patients with symptoms of stable or unstable angina pectoris due to single or multivessel coronary artery disease, who were scheduled to undergo elective PTCA, were considered for inclusion. A total of 5636 patients were screened, of whom 4932 were excluded from the trial because of the criteria listed in Table 1.

Treatment Allocation

The trial was carried out in accordance with the declaration of Helsinki (1973) and its revision in Venice (1983) in 15 participating centers. Written informed consent was obtained from 704 patients who met the selection criteria. Once included, these patients were randomized to double-blind treatment with either ketanserin (maintenance dose, one 40-mg tablet twice daily) or matching placebo. Treatment with oral trial medication was continued until repeat angioplasty at 6 months after the original PTCA. Trial medication was always started at least 1 hour before balloon insertion.

In the first 79 patients, trial medication at the start consisted of an intravenous bolus injection of ketanserin (10 mg given over 3 minutes) followed by an intravenous infusion of 4 mg/h. The application of intravenous trial medication was continued until 1 hour after the last balloon inflation. Patients randomized to placebo received the corresponding solvent volumes in a double-blind fashion. In the subsequent 625 patients, trial medication at the start consisted of an oral tablet (40 mg ketanserin or matching placebo). In all patients, the application of trial medication was resumed 3 to 10 hours after the last balloon inflation with an oral tablet (40 mg ketanserin or placebo). As a mandatory concomitant therapy, acetylsalicylic acid (250 to 500 mg per 24 hours) was started at least 1 hour before balloon insertion and was also continued until follow-up angiography at 6 months. Heparin 10 000 IU was given for the first hour of the procedure to be followed by supplementary heparin at the discretion of the treating physician. When calcium antagonists were already being taken before randomization, they could be continued. Potassium-losing diuretics were only allowed to be taken in combination with potassium-sparing diuretics, such as amiloride or triamterene. Antiplatelet drugs, including nonsteroidal anti-inflammatory drugs other than acetylsalicylic acid and anticoagulants, were not allowed. Trial medications were packed and supplied by the Janssen Research Foundation, which also prepared the randomized plan of drug administration. Randomization was stratified by center.

TABLE 1. Reasons for Exclusion for 3437 of 3902 Screened Patients in 10 of 14 Participating Centers

Previous angioplasty of the same vessel or branches thereof	541 (16%)
Large myocardial infarction within the last 2 weeks (max CK >5×normal)	291 (8%)
Factors making follow-up difficult (no fixed address, etc)	258 (7%)
Participation in another study with any investigational drug (<30 days)	246 (4%)
Informed consent refused	184 (5%)
Use of potassium losing diuretics or hypokalemia (K≤3.5 mmol/L)	168 (5%)
Intended angioplasty of a coronary bypass	155 (5%)
Factors making repeat angiography unlikely	139 (4%)
Intended surgical interventions	41 (1%)
Evolving myocardial infarction (at present CK >2×normal)	35 (1%)
Previous participation in this study	32 (1%)
Indication for (continued) treatment with oral anticoagulants	30 (1%)
Contraindication for treatment with acetyl salicylic acid	28 (1%)
Women who were potentially childbearing	25 (1%)
Contraindication to discontinue preexisting treatment with an antithrombotic agent	24 (1%)
Life expectancy less than 1 year	23 (1%)
Indication for (continued) use of a class Ia, Ic, or III antiarrhythmic drug	17 (<1%)
Documented peptic ulcer or upper gastrointestinal bleeding (<6 months)	15 (<1%)
History of bleeding disorder	11 (<1%)
Severe hepatic disease	10 (<1%)
QT interval exceeding 500 milliseconds in the resting ECG	9 (<1%)
Cerebrovascular accident(s) (<6 months)	4 (<1%)
Under 30 years of age	3 (<1%)
Second- or third-degree heart block	3 (<1%)
Other	1079 (31%)

Angioplasty Procedure and Follow-up Angiography

The angioplasty procedures were left to the discretion of the operator. Multistage procedures (dilatations of different sites during separate procedures) were considered one procedure as long as they took place within 1 week.

For the purpose of the study, three coronary angiograms were obtained in each patient, one just before angioplasty, one immediately after angioplasty, and one at follow-up after discontinuation of the trial medication. If symptoms recurred within 6 months, coronary angiography was carried out earlier. If no definite restenosis was present and the follow-up time was less

than 3 months, the patient was asked to undergo another coronary angiogram at 6 months. To achieve maximal vasodilatation, each angiogram was preceded by either nitroglycerin or isosorbide dinitrate given intracoronarily. Angiograms were recorded in such a way that they were suitable for quantitative analysis by the Coronary Angiography Analysis System (CAAS), using fixed table systems and 35-mm cinefilm at a minimum speed of 25 frames per second. All necessary details of the procedure were recorded in the case record form, and drawings of the segments to be analyzed were made by the investigators. Before the post-angioplasty angiogram, radiopaque guide wires had to be removed to avoid interference with automated edge detection. For calibration purposes, catheter tips were cut off and sent with the cinefilm to the angiographic core laboratory. To standardize the method of data acquisition and to ensure exact reproducibility of post-angioplasty and follow-up angiograms, measures were undertaken as has been described earlier.[29-31] The analyses were performed at the core laboratory; the personnel were blinded to treatment allocation and did not have access to clinical data.

The accuracy and precision of the edge detection procedure of the CAAS system as assessed from cinefilm of contrast-filled acrylate models are −30 and 90 μm, respectively; the variability of the analysis procedure itself in terms of absolute lumen dimension is less than 0.12 mm. The short-, medium- and long-term measurement variability has been assessed from repeated coronary angiographic examination performed 5 minutes, 1 hour, and 90 days apart, respectively. For all studies, the mean difference in absolute diameter was less than 0.13 mm.[32] Since one of the main criticisms of angiography has been the potential measurement inaccuracy and imprecision immediately after balloon angioplasty when disruption of vessel contour is virtually always produced, we have recently analyzed the change in minimal lumen diameter (MLD) observed over a period of 24 hours after PTCA. Post-PTCA accuracy and precision of MLD measurement by the CAAS system are ±0.1 mm and ±0.2 mm, which are eminently acceptable.[33]

The morphology of the stenotic lesions was analyzed and reported according to the Ambrose classification.[34] The TIMI perfusion index was determined by the core laboratory, and the incidence of TIMI 0 and TIMI 1 was reported. The percentages of the stenotic lesions from which a side branch originated are tabulated in Table 2, as well as the number of lesions in which the origin of side branch was involved during balloon inflation. A stenosis was considered to be located in a bend, if, in any nonforeshortened angiographic projection, it appeared that the balloon in position for the dilatation was located in a portion of the vessel that was angulated more than 45° in diastole.[35]

Intracoronary thrombus was defined as the presence of a filling defect within the lumen, surrounded by contrast material seen in multiple projections in the absence of calcium within the filling defect, the persistence of contrast material within the lumen, or a visible embolization of intraluminal material downstream.

All postangioplasty angiograms were examined for the presence or absence of dissection—defined according to modified National Heart, Lung, and Blood Institute criteria—as the presence of angiographically evident intimal or medial damage, presenting either as a small radiolucent area within the lumen of the vessel (tear or flap, type A) or as an extravasation of nonpersisting or persisting contrast medium (type B or C).[36] A dissection was classified as type D in the presence of a spiral-shaped filling defect with delayed distal flow and as type E if a persistent luminal defect with delayed antegrade flow was seen on the final post–coronary angioplasty angiogram. A filling defect accompanied by a total coronary occlusion was classified as a type F dissection.

The absolute values of the MLD and the reference diameter (both in millimeters) were measured using the contrast–empty catheter diameter as a scaling device. In case of total occlusion or lesion with TIMI-1 perfusion, a value of 0 mm was used for the MLD and 100% was used for the percent diameter stenosis. In these cases, the post-PTCA reference diameter was used as the reference diameter pre-PTCA or at follow-up.

Clinical Follow-up

Patients were seen in the outpatient clinic after 1 and 6 months for an interview, a physical examination, a tablet count, and an ECG. At 2 and 4 months, a telephone interview was performed for recording of clinical events and use of trial and concomitant medication. Clinical follow-up status was assessed at 6 months after the procedure either at repeat angiography or at a visit to the outpatient clinic. If this was not possible, the patient was interviewed by telephone. Plasma levels of ketanserin were determined before discharge as well as at 6 months.

End Points

The primary clinical end point of the study was the occurrence of any one of the following: cardiac death, myocardial infarction, or the need for repeat angioplasty or bypass surgery (of the previously dilated sites) between the first balloon inflation and repeat angiography at 6 months (or 6 months calendar time if 6 months repeat angiography was not performed). This combined end point included periprocedural infarctions and emergency bypass surgery. An indication to perform repeat angioplasty or bypass surgery based on findings at 6-month repeat angiography constituted an end point provided that the treating physician could substantiate the decision on the basis of findings at angiography in combination with chest pain and/or electrocardiographic or scintigraphic evidence of myocardial ischemia either at rest or during exercise. Patients in whom no balloon inflation had occurred were left out of the analyses of the clinical end point (n=42). All events that were potential end points were centrally reviewed. End point definitions were as follows: (1) cardiac death: all deaths were considered cardiac unless an unequivocal noncardiac cause could be established; (2) myocardial infarction: the presence of at least two of the following: (a) occlusion of a previously patent coronary artery, (b) prolonged chest pain, (c) a serial enzyme pattern typical of myocardial infarction with at least one cardiac enzyme raised to more than twice the local upper limit for normal, and (d) the development of new Q wave formation; (3) repeat angioplasty: repeat angioplasty after the initial procedure involving at least one of the

TABLE 2. Angiographic Baseline Data of Patients in Intention-to-Treat Analysis

	Ketanserin (N=328)	Placebo (N=330)
No. of lesions	409	407
No. of lesions per patient	1.25	1.23
Description of lesions before angioplasty		
Location of lesion		
RCA	114 (28)	140 (34)
LAD	189 (46)	159 (39)
LCx	103 (25)	98 (24)
LM	1 (<1)	0 (<1)
Type of lesion[29]		
Concentric	170 (42)	179 (44)
Eccentric type IA	31 (8)	26 (6)
Eccentric type IB	81 (20)	77 (19)
Eccentric type IIA	11 (3)	15 (4)
Eccentric type IIB	14 (3)	16 (4)
Multiple irregularities	38 (9)	37 (9)
Tandem lesion	15 (4)	16 (4)
TIMI 0	24 (6)	17 (4)
TIMI I	20 (5)	21 (5)
Side branch in stenosis	155 (38)	160 (40)
Side branch in dilatation site	275 (68)	288 (71)
Relationship to artery bend	103 (25)	110 (27)
Vessel calcified	57 (14)	48 (12)
PTCA procedure		
Total duration of inflation (s)	227±177	229±238
Nominal size of the largest balloon (mm)	2.86±0.42	2.84±0.41
Max inflation pressure (atm)	8.86±2.70	9.00±6.21
Balloon to artery ratio	1.13±0.18	1.10±0.17
Visual assessment of PTCA result		
Grade of perfusion (TIMI score)		
TIMI 0	4 (1)	4 (1)
TIMI I	2 (<1)	3 (1)
TIMI II	8 (2)	14 (3)
TIMI III	388 (96)	385 (95)
Not applicable	1 (<1)	0 (0)
Dissection at the dilated site[31]		
No dissection	276 (68)	251 (62)
Type A	51 (13)	66 (16)
Type B	56 (14)	63 (16)
Type C	17 (4)	16 (4)
Type D	2 (<1)	1 (<1)
Type E	2 (<1)	3 (1)
Type F	2 (<1)	2 (<1)
Thrombus visible		
Before PTCA	14 (3)	12 (3)
After PTCA	16 (4)	6 (1)
Before and after PTCA	2 (<1)	10 (2)

RCA indicates right coronary artery; LAD, left anterior descending coronary artery; LCx, left circumflex artery; LM, left main stem; TIMI, Thrombosis in Myocardial Infarction; and PTCA, percutaneous transluminal coronary angioplasty.

previously dilated lesions; (4) and bypass surgery: emergency bypass surgery or elective coronary bypass surgery involving at least one of the previously dilated lesions (coronary bypass involving other coronary arteries only did not constitute an end point).

The primary angiographic end point of the study was the within-patient change in MLD at the dilated coronary site(s) at follow-up relative to post-PTCA. If a revascularization procedure involving the dilated lesion had been performed before 6-month repeat angiography, the last angiogram obtained before the intervention, if available, was used. In the absence of a 6-month repeat angiogram, the last angiogram obtained within the previous 3 months, if available, was used, provided that no end points had taken place.

For each dilated segment, the MLD was taken as the mean value from multiple matched projections (2.2±0.7 per lesion and orthogonal whenever possible). Within-patient change was defined as the follow-up minus the post-PTCA value. In case more than one segment was dilated (multivessel or multisite procedures), the mean change of all lesions was taken as the angiographic end point.

Secondary end points of the study were: (1) the change in percentage diameter stenosis (100%×[reference diameter minus MLD]/reference diameter) at the dilated site(s) at follow-up angiography relative to baseline, (2) evidence of restenosis at follow-up angiography indicated by a change from <50% stenosis post-PTCA to >50% at follow-up, and (3) the clinical status of each patient at the end of follow-up ranked according to the following ordinal scale (definitions as above): 1, death; 2, myocardial infarction; 3, coronary artery bypass graft (CABG); 4, repeat PTCA; 5, presence of angina pectoris, either exertional (Canadian Cardiovascular Society [CCS] classification 1 or higher) or nonexertional; and 6, none of the above.

Analysis

The main clinical analysis consisted of a single comparison between the trial medication groups of the primary clinical end point, irrespective of the time of its occurrence, involving all randomized patients with the exception of those in whom no balloon inflation had taken place (intention-to-treat analysis). The main analysis of the angiographic data was a comparison of change in lumen diameter from post-PTCA to late follow-up (loss) in patients treated with ketanserin to those treated with placebo, involving all patients of the main clinical analysis with analyzable angiograms (angiographic efficacy analysis). In addition, the primary angiographic end point was analyzed involving those patients who had been compliant until the time of follow-up angiography (per protocol analysis). A patient was judged compliant if at least 80% of the trial medication had been taken and this medication had not been interrupted for more than 14 consecutive days.

An unpaired t test was used for the angiographic and other continuous variables, and a χ^2 test was used for event rates and other discrete factors.[37] Whenever possible, estimates of the magnitude of the trial medication effect (ketanserin relative to placebo) with corresponding 95% confidence intervals are provided. Relative risks are in all cases given as ketanserin relative to placebo.

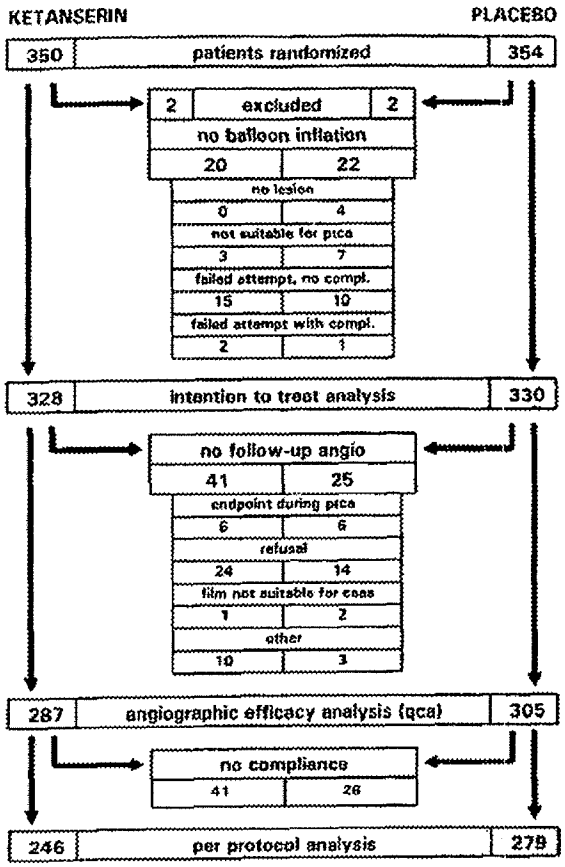

FIG 1. *Patient flowchart in the PARK trial. ptca indicates percutaneous transluminal coronary angioplasty; compl, complication; and qca, quantitative coronary angiography.*

Results

Patient and Baseline Characteristics

Fig 1 shows the patient flowchart. Seven hundred four patients were randomized. Four patients (ketanserin, 2; placebo, 2) were included inappropriately and 42 patients (ketanserin, 20; placebo, 22) did not undergo balloon inflation. The intention-to-treat analysis therefore comprised 658 patients.

Quantitative angiographic follow-up was not available in 66 patients (ketanserin, 41; placebo, 25) for various reasons. The angiographic efficacy analysis thus comprised 592 patients. Finally, 67 patients (ketanserin, 41; placebo, 26) did not fulfill the compliance criteria and were excluded from the per protocol analysis, which thus comprised 525 patients.

Selected demographic and clinical characteristics of the two study groups are shown in Tables 2 and 3. Table 4 shows the clinical events that occurred. Clinical follow-up was obtained for all 658 patients, except for one patient, who was lost to follow-up after the 2-month telephone interview (moved abroad), when no event had occurred.

Clinical End Points

The primary clinical end point occurred in 92 of 328 ketanserin patients and in 104 of 330 placebo patients.

TABLE 3. Clinical Baseline Data of 658 Patients Included in Analysis for Clinical End Points

	Ketanserin (N=328)	Placebo (N=330)
Age (y)		
30-50	79 (24)	87 (26)
51-60	127 (39)	126 (38)
>60	122 (37)	117 (35)
Male sex	263 (80)	264 (80)
Smoking status		
Current	61 (19)	64 (19)
Previous	164 (50)	165 (50)
Never	103 (31)	101 (31)
Diabetes mellitus	38 (12)	36 (11)
Previous myocardial infarction	131 (40)	129 (39)
Previous CABG	15 (5)	13 (4)
Previous PTCA	10 (3)	16 (5)
History of hypertension	103 (31)	131 (40)
History of hypercholesterolemia	132 (40)	156 (47)
History of stroke	8 (2)	8 (2)
History of peripheral vascular disease	22 (7)	25 (8)
Exertional angina		
CCS class I	46 (14)	46 (14)
CCS class II	124 (38)	131 (40)
CCS class III	105 (32)	103 (31)
CCS class IV	31 (9)	27 (8)
No exertional angina	22 (7)	23 (7)
Nonexertional angina	132 (40)	153 (46)
Medication at screening		
Nitrates	173 (53)	183 (55)
Ca antagonists	204 (62)	223 (68)
β-blockers	186 (57)	187 (57)
Monotherapy	108 (33)	96 (29)
Double therapy	127 (39)	154 (47)
Triple therapy	67 (20)	63 (19)
No therapy	26 (8)	17 (5)

CABG indicates coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty; and CCS, Canadian Cardiovascular Society angina classification. Numbers in parentheses are percentages.

The relative risk for the ketanserin group to the placebo group was 0.89, with a 95% confidence interval ranging from 0.70 to 1.13. Fig 2 shows the cumulative distributions of the primary clinical end point over time for both treatment groups. The two curves are not strictly superimposed, indicating that throughout the trial there was a slightly lower cumulative incidence of clinical end points in the group treated with ketanserin as compared with the placebo group.

During the course of the study, 4 patients died (ketanserin, 2; placebo, 2). The cause of death was in all cases cardiovascular (ketanserin: death after 3 months in connection with CABG after various unsuccessful angioplasty procedures; death after an unsuccessful initial procedure and patient refusal of further treatment; placebo: death after 2 weeks due to severe neurological damage after complicated initial PTCA and emergency CABG; and death after 3 months after myocardial infarction due to thrombosis of stent). Nonfatal myocardial infarction was documented in 24 patients (ketanserin, 13; placebo, 11); 49 patients underwent bypass surgery (ketanserin, 21, placebo, 28); and 145 patients underwent repeat PTCA, atherectomy, laser angioplasty, or stent implantation (ketanserin, 69; placebo, 76). At 6-month follow-up, a comparable number of patients in both treatment groups were in each CCS class. Recurrent angina was observed in 242 patients (ketanserin, 111; placebo, 131). Finally, 181 patients (54%) in the ketanserin group and 176 (51%) in the placebo group were event and symptom free at 6-month follow-up.

There were more patients with adverse experiences in the ketanserin group (220 of 328) than in the placebo group (173 of 330). The most frequently reported adverse experiences are summarized in Table 5. Complaints of dizziness and dry mouth appeared to be more common in the ketanserin group than in the placebo group, as was the occurrence of hypotensive episodes. Such adverse experiences could be expected from the known side-effect profile of ketanserin, a compound used for the treatment of hypertension at daily doses of 20 mg bid.[38]

Result of the Angiographic Analysis (Angiographic Efficacy Analysis)

Table 6 summarizes the quantitative angiographic findings in the angiographic efficacy analysis. The loss at follow-up in MLD was 0.27±0.49 mm in the ketanserin group and 0.24±0.52 mm in the control group (treatment effect, 0.03 mm; 95% confidence interval, −0.05 to 0.11 mm). Figs 3 and 4 represent cumulative frequency curves of the MLD and of the loss in MLD observed in both groups.

The restenosis rate per lesion according to the >50% diameter stenosis criterion is 32% in the ketanserin group and 32% in the placebo group, with a relative risk of 0.99 (95% confidence interval, 0.78, 1.25).

Discussion

Rationale for Serotonin Antagonism in the Prevention of Restenosis

Ketanserin is a serotonin $5HT_2$-receptor antagonist. It inhibits serotonin-induced platelet activation and vasoconstriction, but it does not inhibit serotonin-induced vasodilation.[39] In case of damaged endothelium, however, serotonin gains direct access to the smooth muscle cells, causing them to contract.[40] Serotonin is an important mediator in cyclic flow reduction in stenotic and injured arteries.[41] This phenomenon was associated with abundant intimal hyperplasia in the 3 weeks after intimal denudation in animals.[12,42] It was thus surmised that prevention of this phenomenon would directly or indirectly prevent an exuberant wound healing process and the active intimal hyperplasia after wall injury.

Serotonin is released from the dense granules of aggregating platelets. In itself, it is a weak agonist for

TABLE 4. Occurrence During 6-Month Follow-up of Clinical Events and the Primary Clinical End Point

	Ketanserin (N=328)	Placebo (N=330)	RR (95% CI)
Death			
After initial PTCA, before discharge	1	1	
After discharge	1	1	
All	2 (<1%)	2 (<1%)	1.01 (0.14-7.10)
Myocardial infarction			
During initial PTCA	1	3	
After initial PTCA, before discharge	6	4	
After discharge	6	4	
All	13 (4%)	11 (3%)	1.19 (0.54-2.62)
CABG			
Periprocedural	5	6	
After initial PTCA, before discharge	3	6	
<6 mo	9	12	
>6 mo	4	4	
All	21 (6%)	28 (8%)	0.75 (0.44-1.30)
Repeat angioplasty			
Within 1 wk	5	9	
<6 mo	27	28	
>6 mo	37	39	
All	69 (21%)	76 (23%)	0.91 (0.68-1.22)
Primary clinical end point*	92 (28%)	104 (32%)	0.89 (0.70-1.13)

PTCA indicates percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft.

Numbers are patients who experienced at least one of the respective events; <6 mo indicates after 1 week/discharge, before 6-month repeat angiography; >6 mo, subsequent to 6-month repeat angiography.

*See text.

platelet activation, but it enhances the activity of other platelet agonists, such as ADP, thromboxane A_2, catecholamines, and thrombin, by a positive feedback loop. The in vitro evidence with human platelets suggests that serotonin can substantially contribute to strong platelet activation through amplification, a phenomenon that can be blocked by ketanserin.[17] Animal studies have shown that serotonin is released during platelet activation.[43-45] High coronary sinus blood concentrations of serotonin and increased concentrations of serotonin at sites of endothelial injury are seen in in vivo animal models with spontaneous occlusive coronary thrombus formation.[44] The cumulative evidence obtained in animal research suggested that blockade of serotonin 5-HT_2 receptors with ketanserin is as antithrombotic as acetylsalicylic acid in cases in which a modest insult, such as endothelial cell injury, forms the underlying cause of the thrombus; this blockade also appears to be complementary to the blockade of the platelet arachidonic acid pathway by specific inhibition of the cyclooxygenase enzyme in cases with strong platelet activation. Such a strong platelet activation may occur when deep intimal injury forms the underlying cause of the occlusion and escapes a single pharmacological intervention.[12,45-47] Marcos et al[48] found that serotonin-induced vasospasm was potentiated by indomethacin in isolated perfused bovine coronary arteries. This finding may have important clinical consequences for patients undergoing coronary angioplasty, where the endothelium is damaged, platelets accumulate, and prostanoid synthetic activity is likely compromised.[48] Sigal and coworkers[49] observed that proximal but not distal coronary spasm after balloon

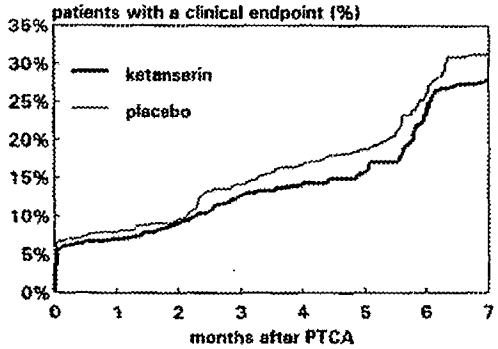

FIG 2. *Cumulative distribution curve of clinical end points. PTCA indicates percutaneous transluminal coronary angioplasty.*

TABLE 5. Adverse Events In Trial Medication Groups

	Ketanserin	Placebo	All	
IV loading dose	**(n=37)**	**(n=39)**	**(n=76)**	***P***
Upper abdominal complaint	3 (8)	4 (10)	7 (9)	1.00
Dizziness	5 (14)	0 (0)	5 (7)	.06
Bleeding event	6 (16)	6 (15)	12 (16)	1.00
Hypotensive event	9 (24)	4 (10)	13 (17)	.19
Dry mouth	7 (19)	2 (5)	9 (12)	.13
Arrhythmia	1 (3)	3 (8)	4 (5)	.65
Other	9 (24)	8 (21)	17 (22)	.90
None	12 (32)	20 (51)	32 (42)	.15
Oral loading dose	**(n=291)**	**(n=291)**	**(n=582)**	
Upper abdominal complaint	43 (15)	34 (12)	77 (13)	.33
Fatigue	49 (17)	35 (12)	84 (14)	.13
Dizziness	47 (16)	19 (7)	66 (11)	<.001
Bleeding event	22 (8)	26 (9)	48 (8)	.65
Hypotensive event	19 (7)	9 (3)	28 (5)	.08
Dry mouth	22 (8)	9 (3)	31 (5)	.03
Arrhythmia	22 (8)	10 (3)	32 (6)	.045
Other	115 (40)	102 (35)	217 (37)	.30
None	96 (33)	137 (47)	233 (40)	.001
All patients	**(n=328)**	**(n=330)**	**(n=658)**	
Upper abdominal complaint	46 (14)	38 (12)	84 (13)	.40
Fatigue	49 (15)	35 (11)	84 (13)	.12
Dizziness	52 (16)	19 (6)	71 (11)	<.001
Bleeding event	28 (9)	32 (10)	60 (9)	.70
Hypotensive event	28 (9)	13 (4)	41 (6)	.023
Dry mouth	29 (9)	11 (3)	40 (6)	.005
Arrhythmia	23 (7)	13 (4)	36 (5)	.12
Other	124 (38)	110 (33)	234 (36)	.26
None	108 (33)	157 (48)	265 (40)	<.001

Multiple events per patient are counted under the various headings. Numbers in parentheses are percentages. *P* value from continuity-adjusted χ^2 test.

dilatation can be prevented by a serotonin 5-HT_2 antagonist. Golino and coworkers[50] observed that intracoronary infusion of serotonin in patients with normal coronaries at cardiac catheterization markedly dilated the epicardial coronary arteries and increased coronary blood flow and that these effects were enhanced by the administration of ketanserin, whereas the serotonin caused profound vasoconstriction in the distal portions of coronary arteries with atherosclerosis.

Serotonin has also been shown to stimulate DNA synthesis in vascular smooth muscle cells in vitro.[19,51] In vitro experiments show the stimulation of mitogenesis, migration, and retraction of vascular smooth muscle cells after serotonin exposure. In low concentrations, it substantially enhances the mitogenic response of these cells to PDGF[19] as well as the proliferation of cultured fibroblasts and matrix synthesis.[15]

This mitogenic effect of serotonin can be blocked by ketanserin. When vascular smooth muscle cells are allowed in vitro both to produce an extracellular matrix and intercellular junctions, addition of fresh serum stimulates the coordinate retraction of this matrix. Serotonin present in high concentrations (10^{-5} mol/L) is important in this response, since (1) only serotonin (of all tested agents) induced a comparable degree of retraction and (2) the serum response can be blocked by 5-HT_2 serotoninergic receptor blockers. Ketanserin also decreased the collagen content in an in vivo granuloma model, where a similar cell type has been implicated.[24]

In view of the earlier mentioned properties of ketanserin, its administration could have a protective effect on early and late restenosis of coronary arteries after coronary angioplasty.

Dosage

The selection of the intravenous starting dose of ketanserin was based on the finding that an infusion rate of 0.1 mg/min appeared to be the lowest dose that was

TABLE 6. Quantitative Analysis: Intention-to-Treat Analysis, Lesion Values Averaged per Patient

	Ketanserin (N=287)	Placebo (N=305)	Significance
Reference diameter (mm)			
Before angioplasty	2.58±0.51	2.67±0.53	
After angioplasty	2.65±0.50	2.69±0.49	NS
At follow-up	2.66±0.58	2.76±0.52	$P=.02$
Minimal luminal diameter (mm)			
Before angioplasty	0.97±0.41	0.97±0.39	
After angioplasty	1.70±0.37	1.69±0.34	NS
At follow-up	1.43±0.62	1.44±0.58	NS
Difference in minimal luminal diameter (mm)			
Acute gain	0.73±0.41	0.72±0.39	NS
Late loss	0.27±0.49	0.24±0.52	NS
Net gain	0.46±0.60	0.47±0.56	NS
Percentage stenosis			
Before angioplasty	62±15	63±14	
After angioplasty	35±11	37±9	$P=.04$
Follow-up	47±19	48±19	NS

Acute gain: minimal luminal diameter (MLD) after percutaneous transluminal coronary angioplasty (PTCA) minus MLD before PTCA; late loss: MLD after PTCA minus MLD at follow-up; net gain: MLD at follow-up minus MLD before PTCA.
NS indicates not significant at the $P=.05$ level.

maximally effective in inhibiting ADP-induced or adrenaline-induced platelet aggregation ex vivo.[52] This dose was subsequently used in a pilot study in patients with coronary artery stenosis, where ketanserin administration was started after balloon angioplasty and continued for 12 hours. In the latter study, Klein and coworkers[52] were able to show a reduction in early (after 24 hours) but not in late (after 4 to 9 months) restenosis after balloon angioplasty.

In the design of the present trial, it was deemed advantageous to start the ketanserin infusion before the balloon inflation and its potentially disruptive effects on the arterial wall, leading to local platelet deposition and stenosis. For practical reasons, infusion of trial medication could not be initiated earlier than 1 hour before the insertion of the catheter. To ensure that enough ketanserin would be "on board" during the procedure, pharmacokinetic simulations were carried out that indicated the necessity for a loading dose.

In the early phase of the trial, patients were treated according to the above dosage schedule, and some cases of periprocedural hypotension and bradycardia were observed. Due to an apparently uneven distribution of cases among the treatment groups (hypotension was reported in 7 of 37 [19%] patients in the ketanserin group and in 3 of 39 [8%] patients in the placebo group), the Ethics and Safety Committee

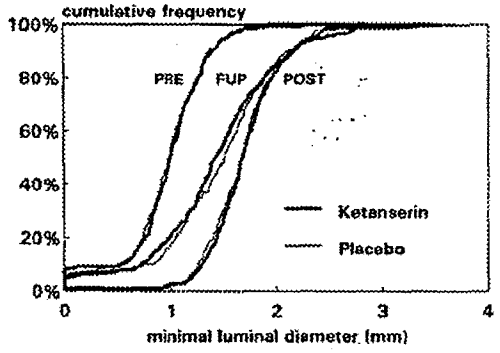

FIG 3. *Cumulative distribution curve of the minimal luminal diameter before (PRE) and after (POST) percutaneous transluminal coronary angioplasty and at 6-month follow-up (FUP) in both treatment groups.*

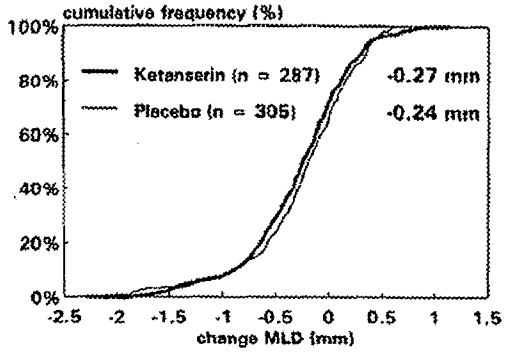

FIG 4. *Cumulative distribution curve of the change in minimal lumen diameter (MLD) at follow-up respective of post-percutaneous transluminal coronary angioplasty MLD (late loss).*

suggested to consider an adjustment of the dosage schedule. It was thereupon decided to leave out the intravenous administration and, instead, start with an oral tablet that was taken 1 hour before catheter insertion. This would yield plasma levels during balloon inflation that were at least similar to those obtained in the original intravenous schedule. The protocol was amended accordingly, and vasovagal reactions were found to be rare in the rest of the patients.

The oral maintenance dose was chosen such that plasma ketanserin levels would exceed 10^{-8} mol/L, which is the concentration that elicits virtually complete inhibition of serotonin-induced platelet aggregation ex vivo. This requirement is usually met after oral ketanserin administration at the recommended antihypertensive dose (ie, 20 or 40 mg bid). Nadir plasma levels, however, may not always exceed this threshold after the lower dose, but they did after the higher (personal communication by F. De Clerck). It was, therefore, decided to select the 40 mg bid regimen as the maintenance dose in this trial.

Trial Design

The mechanism of action of ketanserin implied that it would prevent both acute occlusion due to platelet aggregation–induced thrombus formation and late restenosis due to platelet aggregation–induced hyperplasia. As a consequence, trial medication was started before the procedure, ie, before wall injury occurred. This had major consequences for the definition of the clinical end point. On the one hand, all failures between the first balloon inflation and the end of the procedure could have been influenced by the trial medication and were therefore counted as clinical end points. On the other hand, as the aim of this trial was to study the effect of ketanserin on the inhibition of the neointimal hyperplasia after balloon wall injury, it seemed reasonable to exclude those patients from the analysis of the main clinical end point in whom no balloon inflation had occurred.

At this stage of the development of the therapeutic principle, we considered it necessary to establish the mechanism of action by direct angiographic observation of the encroachment of the lumen over a period of 6 months. For this reason, the trial was designed to have two major analytic thrusts: a comparison of clinical end points and a comparison of the loss in lumen diameter. This, however, required a compromise for the power of the trial. Based on the angiographic principle, with 300 patients per trial medication group, the trial had more than 95% power to statistically distinguish a loss in lumen diameter of 0.40 mm under placebo from 0.25 mm under ketanserin (the standard deviation of the loss in lumen diameter is known to be around 0.50 mm). Against this, the trial had only marginal power to detect differences in the rates of the primary clinical end point: with 300 patients, the power to distinguish an event rate of 25% under placebo from 15% under ketanserin was only 85%. At the end of the trial, the loss in lumen diameter under placebo turned out to be 0.24 mm. This unanticipated finding, however, eroded the power of the angiographic analysis to some extent: a similarly (40%) sized reduction from 0.25 mm to 0.15 mm could only be detected with 75% power. Over the last few years, it has become apparent that the loss in MLD in different trials could vary considerably, since in the placebo groups of the most recent trials, the loss in MLD ranged from 0.24 to 0.36 mm, although at this stage we do not have a clear explanation for this intertrial variability.[53-55] It is surmised that differences in baseline demographic data could have affected the mean loss in MLD at follow-up. Factors such as the percentage of recruited patients in CCS class 4, patients with recent onset of angina, unstable patients, diabetics, and the frequency of total occlusion apparently have a major impact on the loss in MLD at follow-up. In a recent editorial in *Circulation*, Popma, Califf, and Topol[56] recommended that in future trials high-risk patients not be excluded, so as to avoid a misrepresentation of the population of typical patients undergoing angioplasty.

One of the consequences of having systematic angiographic follow-up at 6 months is that it thwarts the natural occurrences of clinical end points (reinterventions). As a consequence, all indications for a revascularization procedure that had been triggered by the 6-month repeat angiography were counted as end points, provided that the indication was also substantiated by anginal symptoms or positive findings at exercise testing.

For the definition of the primary clinical end point, we chose a combination of the major untoward clinical events: death, myocardial infarction, referral for coronary bypass surgery, or an indication for repeat angioplasty as the major clinical end point. The advantages are obvious, as was recently indicated in the above-mentioned editorial[56]: the definition of the clinical end point is based on so-called hard criteria, the end point is evaluable in all randomized patients, and the end point leads to simple effect estimates with corresponding 95% confidence intervals.

The process of luminal narrowing after coronary balloon angioplasty is approximately normally distributed, with few lesions showing regression, most of the lesions showing no change, and a considerable amount of the lesions showing progression. Restenosis can thus be viewed as the tail end of a near-Gaussian distribution, with some lesions crossing a more or less arbitrary angiographic cutoff point rather than a separate disease entity that occurs in some lesions but not in others. For comparison of the angiographic efficacy of pharmacological agents, we therefore recommend the use of change of MLD as an end point rather than restenosis rate.[57,58] Statistically, the quantitative outcome can be evaluated with less than a third of the number of patients required for assessment of the categorical outcome. This is indeed logical because categorical end points do not take full advantage of the available information, discard quantitative information, and therefore result in loss of statistical power. For the angiographic analysis, two approaches exist: a per-lesion or a per-patient analysis. In the latter, the patient is taken as the unit of analysis by averaging lesion values per patient. Statistically, a per lesion analysis is appropriate only if the changes per lesion within patients act independently.[37] As we could not guarantee that this would be the case, we chose the more conservative per-patient analysis.[59]

Quantitative Angiography Versus Clinical Events as Primary End Point

The primary goal of a restenosis prevention trial is the improvement in short- and long-term clinical outcome of patients having undergone a PTCA procedure. It is assumed that the improvement in clinical outcome is related to an anatomic phenomenon, namely the prevention of the recurrence of the stenosis in the treated vessel.[54] Therefore, in this type of trial, testing pharmacological compounds with possible anti-ischemic or antianginal effects unrelated to the postinjury hyperplasia, the clinical outcome might be misleading and obscure the reason for the observed improvement.

In the present study, there was a trend toward a lower incidence of revascularization procedures (repeat PTCA, CABG) in the ketanserin group (77 versus 95 in the control group). At the same time, there was also a difference in the loss in MLD from post-PTCA to follow-up, with the larger loss in MLD observed in the ketanserin group when compared with the placebo group. Although not statistically significant, these contradictory trends raise a question regarding the interpretation of the outcome of the study. If there is evidence of a lesser degree of angina, better functional performance, or as in this case, less need for repeat PTCA or CABG, this implies that the treatment may have an antianginal or anti-ischemic effect that is mediated by some other mechanism than modulation of the proliferative process in the stenotic area.

Indeed, ketanserin has a potential for accounting for such an effect. It has been demonstrated by Golino et al[50] that patients with angina pectoris develop marked coronary vasoconstriction in response to intracoronary injection of serotonin and that this effect was abolished after administration of ketanserin. This may have clinical significance, since coronary vasospasm and vasoconstriction play an important pathogenic role not only in variant angina but also in a wide spectrum of ischemic heart diseases such as effort angina, unstable angina, and acute myocardial infarction. There are also results suggesting that ketanserin promotes coronary blood flow by improving rheological parameters. For example, it has been shown that the deformability of erythrocytes increases[60] and that there is a decrease in whole blood viscosity.[61] Finally, a potentially antianginal mechanism may be mediated by the arteriolar dilating effect of ketanserin resulting in left ventricular unloading. These considerations are speculative, and it must be pointed out that no significant differences in blood pressure and heart rate (two major determinants of the myocardial oxygen demand) were recorded at 1-month follow-up and subsequently, although an initial significant decrease in blood pressure was observed immediately before and after the PTCA procedure in the group treated with ketanserin.

Possible Explanations for the Lack of Effect of Ketanserin in This Trial

As patients were treated with ketanserin only 1 hour before PTCA, the question arises if a longer pretreatment phase might have been more effective. However, pharmacokinetic simulations with the intravenous loading dose as well as studies with oral dosing had shown enough ketanserin to be "on board" during the procedure to ensure adequate platelet aggregation inhibition.[62-64]

Similar numbers of clinical end points in each arm of the trial were encountered in the periprocedural phase. It might be inferred from these results that the preventive administration of isosorbide dinitrate at the time of the procedure had such a beneficial effect that the cardioprotecting effect of ketanserin was masked. Similarly, the administration of aspirin might have masked the potential antiserotonin effect of ketanserin in the treated group, both compounds being similarly potent in terms of platelet aggregation antagonism,[65] so that the additional cardioprotective effect of ketanserin could no longer be detected.

In general, the speculations mentioned before raised the question of the potential benefit of other forms of synergy. At the time of the design of the trial, it was reported that a serotonin antagonist used in synergy with a thromboxane blocker was more potent than each drug by itself.[45,66,67] Further investigation of serotonin antagonist in synergy with a therapeutic agent acting on associated mechanisms of restenosis might be worth pursuing. On the other hand, since there are multiple redundant metabolic pathways involved in the process of platelet aggregation and adhesion, it seems illusive and unrealistic to try to block or selectively inhibit all these multiple metabolic pathways. It has been suggested that the final pathway in the activation and exposure of the platelets could be ultimately interrupted by selectively blocking the glycoprotein receptor IIb-IIIa on the membrane of the thrombocyte.[68] But even then, as has been put forward, an antagonist of the glycoprotein receptor would not prevent the deposition of a single layer of platelets. Since the mitogenic factors are released from the α-granules of the adherent platelets, the blockade of this final pathway would also be insufficient to prevent the initiation of the restenosis phenomenon.

As far as the antiproliferative effect of ketanserin is concerned, it has been shown that the platelet-derived 5-HT stimulates migration and proliferation of vascular smooth muscle cells[19,22-25] and promotes the synthesis of collagen and proteoglycans by isolated cells.[24,26,27] The potential role of this monoamine in neointima formation in reaction to balloon-induced damage of carotid arteries in rats with the use of pharmacological treatment with ketanserin has been studied recently.[69] The results suggested that serotonin in this rat model contributed to some extent to the neointima formation after vascular injury but certainly was not a dominant mediator in this respect. The absence of effect of ketanserin in the present trial may indicate that in humans this monoamine should add even less to the complex biology of late restenosis. Another possible explanation is that, although it might contribute substantially, there may be other mediators responsible, so that inhibitors of multiple mediators are required. Considering the rate of adverse events documented in the present study and the absence of clinical benefit, however, we are not recommending the isolated use of this drug for prevention of restenosis. However, further study in combination with other synergetic compounds should not be disregarded.

Appendix

Steering Committee

P.W. Serruys, MD (Chairman); W. Klein, MD; J. Willerson, MD; J. Pool, MD; D. Jackson, MD; R. Van Gool, MD; J. Symoens, MD.†

Ethics and Safety Committee

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Critical Event Committee

F. ten Cate, MD; E. Schroeder, MD; W. Wijns, MD; J. Deckers, MD; B. De Bruyne, MD.

Park Study Group: Participating Centers and Investigators

The following institutions and investigators participated in PARK. The number of patients enrolled at each center is given in parentheses.

Freie Universität Berlin, Univerisätsklinikum Rudolf Virchow/Charlottenburg, Berlin, Germany (95): W. Rutsch, MD, Principal Investigator.

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Onze Lieve Vrouw Ziekenhuis, Cardiovascular Center, Aalst, Belgium (89): G.R. Heyndrickx, MD, Principal Investigator; B. De Bruyne, MD; P. Nellens, MD; M. Goethals, MD; P. Goemaere, RN.

Med Clin I, Sahlgrenska Hospital, Göteborg, Sweden (70): H. Emanuelsson, MD, Principal Investigator; K. Selin, MD.

University of Leeds, England (69): S.G. Ball, MD, Principal Investigator; A.F. Mackintosh, MD; M.R. Rees, MD.

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Emory University, Atlanta, Ga (34): H.A. Liberman, MD, Principal Investigator.

University of Texas Medical School, Houston (28): J.T. Willerson, MD, Principal Investigator; A.V. Adyanthaya, MD; D.B. Cassidy, MD; K.L. Gould, MD; R. Smalling, MD; H.V. Anderson, MD.

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Henry Ford Hospital, Detroit, Mich (25): F. Khaja, MD, Principal Investigator; J. Brymer, MD; S. Goldstein, MD; P. Kraft, MD; T. McFarland, MD.

Emory University, Atlanta, Ga (14): R.W. Alexander, MD, Principal Investigator; S.B. King, MD; H.A. Liberman, MD; D.C. Morris, MD.

Beth Israel Hospital, Boston, Mass (11): D. Baim, MD, Principal Investigator; M.J. Levine, MD; G. Reis, MD.

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Janssen Research Foundation

J. Symoens, MD; R. Van Gool, MD; G. Sieben; A.G. Dupont, MD. Trial Monitors: B. Bohanek (USA); R. Van Gool, MD; H.W. Scheijgrond; S. Versele (Belgium), H.P. Benn, MD (Germany); J.C. van Oene, PhD (The Netherlands); B. Lundgrea, PhD (Sweden); M.B. O'Donovan (United Kingdom); W. Pfeffer, MD (Austria).

Study Directors

J. Pool, MD, for Cardialysis and J. Symoens, MD,† and R. Van Gool, MD, for the Janssen Research Foundation.

†Dr Symoens died during the course of the trial.

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Prevention of Restenosis After Percutaneous Transluminal Coronary Angioplasty With Thromboxane A_2–Receptor Blockade

A Randomized, Double-Blind, Placebo-Controlled Trial

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Background. **GR32191B is a novel thromboxane A_2–receptor antagonist with potent antiaggregational and antivasoconstrictive properties. We have conducted a randomized, double-blind, placebo-controlled trial to study its usefulness in restenosis prevention.**

Methods and Results. **Patients received either GR32191B (80 mg orally before angioplasty and 80 mg/day orally for 6 months) or 250 mg i.v. aspirin before angioplasty and placebo for 6 months. Coronary angiograms before angioplasty, after angioplasty, and at 6-month follow-up were quantitatively analyzed. Angioplasty was attempted in 697 patients. For efficacy analysis, quantitative angiography at follow-up was available in 522 compliant patients (261 in each group). Baseline clinical and angiographic parameters did not differ between the two treatment groups. The mean difference in coronary diameter between postangioplasty and follow-up angiogram (primary end point) was −0.31±0.54 mm in the control group and −0.31±0.55 mm in the GR32191B group. Clinical events during 6-month follow-up, analyzed on intention-to-treat basis, were ranked according to the highest category on a scale ranging from death (control, six; GR32191B, four) to nonfatal infarction (control, 22; GR32191B, 18), bypass grafting (control, 19; GR32191B, 22) and repeat angioplasty (control, 52; GR32191B, 48). No significant difference in ranking was detected. Six months after angioplasty, 75% of patients in the GR32191B group and 72% of patients in the control group were symptom free.**

Conclusions. **Long-term thromboxane A_2–receptor blockade with GR32191B does not prevent restenosis and does not favorably influence the clinical course after angioplasty.** (*Circulation* **1991;84:1568–1580**)

Percutaneous transluminal coronary angioplasty (PTCA) is increasingly being used as an alternative to coronary artery bypass graft surgery in patients with coronary artery disease. Although major improvements in angioplasty techniques have resulted in a high initial success rate, the late restenosis rate of 20–40% still limits the long-term benefit of the procedure.[1–5] For multivessel angioplasty, the restenosis percentage is even higher.[6] It is well known that restenosis after balloon angioplasty is a time-related phenomenon, occurring in the first months after balloon angioplasty.[5,7] Only very rarely does restenosis present itself more than 6 months after coronary angioplasty[8,9]; therefore, the follow-up period has been limited to the first six months after angioplasty in the current trial.

Deendothelialization and vascular disruption at the angioplasty site expose vessel wall smooth muscle cells and collagen directly to blood. This causes platelet adhesion, platelet aggregation, and activation of the clotting cascade. In addition, platelets may

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also activate leukocytes to release vasoconstrictor leukotrienes. These effects appear to be thromboxane mediated as inhibition of thromboxane reduces leukocyte activation.[10] Adhesion and aggregation of platelets at the postangioplasty plaque can lead to an early occlusion within the first 48 hours after angioplasty. Over the long term, platelet- and monocyte-derived growth factors stimulate smooth muscle cell proliferation, leading to the fibroproliferative reaction of the vessel wall in the first months after balloon angioplasty.[11-13] Apart from the proliferation process, organization of mural thrombi may also be the cause of restenosis.[14,15] Early platelet aggregation thus appears to play a pivotal role in the occurrence of postangioplasty thrombotic occlusion and the restenosis process.[16]

Thromboxane A_2 (TXA_2) is a potent platelet aggregational agent and vasoconstrictor released from activated platelets. Beyond the platelet-activating effect, TXA_2 also appears to have a more direct effect on vascular smooth muscle cell proliferation. Using primary cultures of smooth muscle from rat aorta, Hanasaki et al[17] demonstrated a mitogenic effect of thromboxane on smooth muscle cells, which occurs through binding to its specific receptor and may be suppressed by thromboxane-receptor blockade,[17] a promising approach to the inhibition of the effects of TXA_2.[18] TXA_2-receptor blockade prevents the deleterious actions of TXA_2 while sparing the beneficial synthesis of prostacyclin. GR32191B has been shown to be a potent and specific TXA_2-receptor–blocking drug that antagonizes the proaggregatory, vasoconstrictor, and bronchoconstrictor actions of TXA_2, as well as those of agents that act indirectly via TXA_2, such as collagen and arachidonic acid, and agents that directly stimulate the receptor, such as prostaglandin H_2 and the TXA_2 mimetic U-46619.[18,19] Although not affecting platelet adhesion, it potently inhibits the aggregation of platelets onto damaged blood vessels.[18,20] This property, together with the ability of the compound to inhibit the platelet-release reaction, indicates a potential clinical use of GR32191B in reducing early thrombotic events, late intimal hyperplasia, and subsequent restenosis after coronary angioplasty. The present multicenter, randomized, double-blind, placebo-controlled trial (Coronary Artery Restenosis Prevention on Repeated Thromboxane-Antagonism [CARPORT]) was carried out to evaluate the role of GR32191B in the prevention of late restenosis after PTCA.

Methods

All patients with angina and angiographically proven coronary artery disease who were scheduled for angioplasty were considered for inclusion at one of six participating centers (see "Appendix"). The trial was carried out according to the declaration of Helsinki, and specific exclusion criteria are given in Table 1. A screening log was maintained in two centers. At these two centers, 1,614 patients were screened from December 1987 through June 1989, and 72% were excluded (Table 1).

TABLE 1. Reasons for Exclusion for 1,318 of 1,614 Screened Patients at Two of Six Participating Centers

Reason	n	Total (%)
Insufficient lead-in time*	235	18
Use of platelet-inhibiting or nonsteroidal anti-inflammatory drugs within 7 days preceding the study	352	27
Refusal to participate and/or undergo 6-month recatheterization	364	28
Currently taking oral anticoagulant drugs	119	9
Angioplasty for restenosis	105	8
Acute myocardial infarction within 2 weeks preceding angioplasty	52	4
Bypass graft dilatation	39	3
History of obstructive airway disease	26	2
History of peptic disease or upper gastrointestinal bleeding	19	1
Previous participation in the trial	2	0.2
Severe other disease	6	0.5
Participation in another trial	6	0.4
History of intolerance to aspirin	1	0.1
Less than 21 years old	1	0.1
Pregnant woman or woman likely to become pregnant during study	0	0
Total	1,318	100

*Urgent referrals outside working hours.

Randomization and Treatment Protocol

Randomized, double-blind trial medication was allocated by telephone after the patient had been registered at the central allocation service. Trial medication consisted of either GR32191B for 6 months or control treatment with one dose of aspirin, followed by matching placebo.

One hour before angioplasty, patients allocated to GR32191B received 4 tablets of 20 mg GR32191B orally and an intravenous injection of a physiological salt solution. Patients allocated to control treatment received 250 mg i.v. acetylsalicylic acid and 4 placebo tablets. In addition to trial medication, all patients received a bolus of 10,000 units i.v. heparin at the beginning of the procedure. After two hours, 5,000 units/hr was given for as long as the procedure continued. Also, all patients received 10 mg nifedipine every 2 hours for the first 12 hours and 20 mg slow-release nifedipine tablets thereafter every 8 hours up to the second day after angioplasty.

In those patients in whom angioplasty was successful, either 40 mg GR32191B twice daily or placebo was started in the evening and continued until the end of follow-up. The final dose of trial medication was taken 1 hour before the follow-up angiogram. In addition, all participants were provided with paracetamol in 500-mg tablets for use as analgesic and were asked to avoid acetylsalicylic acid or nonsteroidal anti-inflammatory drugs while on trial medication.

Trial medication and paracetamol were packaged and supplied by Glaxo Group Research, which also prepared the random plan. Randomization was stratified by center.

Angioplasty Procedure and Follow-up Angiography

Coronary angioplasty was performed with a steerable, movable guide wire system via the femoral route. Choice of balloon type and brand as well as inflation duration and pressure were left to the operator. For the purpose of the study, three coronary angiograms were obtained in each patient—one just before angioplasty, one immediately after angioplasty, and one at follow-up. Angiograms were recorded in such a way that they were suitable for quantitative analysis by the coronary angiography analysis system (CAAS), using fixed-table systems and 35-mm cinefilm at a minimum speed of 25 frames/sec. All necessary details of the procedure were recorded in the case record form, and drawings of the segments to be analyzed were made by the investigators. Before the postangioplasty angiogram, radiopaque guide wires had to be removed to avoid interference with automated edge detection. For calibration purposes, catheter tips were cut off and sent with the cinefilm to the angiographic core laboratory. To standardize the method of data acquisition and to ensure exact reproducibility of postangioplasty and follow-up angiograms, measures were undertaken as has been described earlier.[5,21,22] A qualitative assessment of certain lesion characteristics was performed (see Table 2). Intracoronary thrombus was defined as the presence of a filling defect within the lumen, surrounded by contrast material seen in multiple projections in the absence of calcium within the filling defect, the persistence of contrast material within the lumen, or a visible embolization of intraluminal material downstream. Haziness was defined as a small radiolucent area within the lumen of the vessel disappearing with the passage of contrast material (type A dissection according to Dorros et al[23]). Intimal tear was defined as a filling defect within the lumen and dissection as contrast appearing outside the lumen, disappearing or persisting with the passage of contrast material (types B and C dissections according to Dorros et al[23]).

Follow-up Evaluation

After successful angioplasty, defined as at least one lesion successfully dilated (i.e., less than 50% diameter stenosis on visual inspection after the procedure) as judged by the investigator, patients returned to the outpatient clinic after 3 weeks and 3, 6, and 7 months for an interview, a physical examination, laboratory tests, a tablet count, and, except for the 6- and 7-month visits, a new supply of trial medication. Patients with an unsuccessful angioplasty discontinued trial medication and received the standard medical care. The follow-up clinical status of all patients, irrespective of PTCA success, was assessed 6 months after the procedure. In one of the participating centers (Rotterdam), platelet aggregation tests, using ADP and U-46619 (a TXA_2 mimetic) as aggregants, were carried out to assess pharmacological activity of the drug. At 6-month follow-up, 1–4 days before angiography, a symptom-limited exercise test was performed on a bicycle ergometer according to two different protocols. In Berlin, the test was performed with the patient in a supine position, starting with a work load of 25 W, which increased by 25 W every 2 minutes. In the other clinics, the test was performed with the patient in a sitting position, starting with a work load of 20 W, which was increased by 20 W every 1 minute. Exercise was continued until anginal symptoms, a drop in systolic blood pressure, severe arrhythmia, or a ST depression of more than 1 mm occurred. A 12-lead electrocardiogram was recorded during exercise and recovery. ST changes were measured 80 msec after the J point. Horizontal or downsloping ST segment depression associated with anginal symptoms was considered a positive response to the stress test. The follow-up coronary angiogram was performed at the 6-month visit. If symptoms recurred within 6 months, coronary angiography was carried out earlier. If no definite restenosis was

Table 2. Angiographic Baseline Data of Compliant Patients With Quantitative Angiographic Follow-up

	Control (n=261)		GR32191B (n=261)	
	n	%	n	%
Lesions (n)	320		316	
Lesions per patient (n)	1.23		1.21	
Vessels dilated				
LAD	167	52	146	46
RCA	90	28	99	31
LCx	63	20	71	23
Calcified lesion	19	6	32	10
Discrete	242	76	239	76
Asymmetry	133	42	134	42
Total occlusion	12	4	10	3
Tandem lesion	25	8	24	8
Side branch in stenosis	99	31	78	25
Side branch in dilatation site	178	56	193	61
Inflation duration (seconds)	138±92		133±90	
Maximum inflation pressure (atm)	9±2		9±2	
Balloon-to-artery ratio	1.10±0.22		1.06±0.22	
Thrombus visible after angioplasty	7		12	
Dissection	49	15	46	15
At balloon site	44		39	
Proximal of balloon	1		1	
Distal of balloon	4		6	
Intimal tear	37	12	26	8
Haziness	45	14	56	18

LAD, left anterior descending coronary artery; RCA, right coronary artery; LCx, left circumflex artery.

present and the follow-up time was less than 4 months, the patient was asked to undergo another coronary arteriogram at 6 months.

Quantitative Angiography

All cineangiograms were analyzed using the CAAS system, which has been described in detail.[24–26] A computer-derived reconstruction of the original arterial dimension at the site of obstruction (assuming there is no disease present) is used to define the interpolated reference diameter. The absolute values of the stenosis diameter as well as the reference diameter are measured by the computer using the known contrast catheter diameter as a scaling device. Because the algorithm is not able to measure total occlusions, a value of 0 mm was substituted for the minimal lumen diameter and 100% for the percent diameter stenosis. In these cases, the postangioplasty reference diameter was substituted for the reference diameter before angioplasty. In contrast, for a totally occluded vessel at follow-up angiography, a value was not substituted, so that the change in reference diameter from after angioplasty to follow-up was only calculated when an actual measurement was available.

Balloon-to-artery ratio was defined as the ratio of the mean balloon diameter measured in a single nonforeshortened projection and the reference diameter of the dilated segment in the same projection.

Assay of GR32191B and Platelet Aggregation Tests

Plasma samples of patients allocated to active drug treatment were taken before first drug intake and approximately 1 hour afterward. These samples were analyzed for GR32191B by high-performance liquid chromatography with fluorescence detection after solid-phase extraction on an advanced automated sample processor.

For the aggregation tests, blood was drawn from the patient by venipuncture. Nine parts of blood were mixed with 1 part 0.13 M sodium citrate solution. The blood was then centrifuged (15 minutes at 200*g* at room temperature), and the supernatant platelet-rich plasma (PRP) was carefully removed using a plastic Pasteur pipette and transferred to a separate plastic tube. The remaining blood was centrifuged for 10 minutes at 2,000*g* at room temperature to obtain platelet-poor plasma (PPP). PPP was then added to PRP to obtain PRP with a platelet count of 200×10^9 platelets/l. The PRP was stored at room temperature in full, capped tubes (contents, 5 ml) for 30–90 minutes. Aggregation was performed in a Payton twin-channel aggregometer at 37°C with a stirring speed of 900 rpm. Maximum and minimum light transmission was set up using PPP and PRP, respectively. Samples of 400 μl PRP were incubated in the aggregometer for 3 minutes at 37°C, and 40 μl of either the TXA_2 mimetic U-46619 (final concentration, 1 μM) or ADP (final concentration, 10 μM) was added. Aggregation was allowed to proceed to its maximum or a period of 5 minutes was allowed, whichever was longer. Aggregation was expressed as the peak response and represented in millimeters.

End Points

The primary end point of the present study was the within-patient change in minimal lumen diameter as determined by quantitative angiography after PTCA and at follow-up. Post-PTCA values were obtained from the last post-PTCA angiogram made before withdrawal of the guide catheter. The initial procedure were considered finished when the guide catheter was removed. In case evolution of the clinical condition required repeat PTCA (with reinsertion of guide catheter), the angiogram made before repeat balloon inflations was used to obtain follow-up values, regardless of the timing of repeat PTCA (hours, days, or weeks). Otherwise, the follow-up angiogram made according to protocol was used. For each dilated segment, the post-PTCA and follow-up minimal lumen diameters were taken as the mean values from multiple matched projections. Within-patient change (i.e., the primary end point) was defined as the follow-up minus the post-PTCA value. In case more than one segment was dilated (multivessel or multisite procedures), the change in minimal luminal diameter per patient was calculated as the average of the different lesions. Secondary end points were clinical events believed to be related to restenosis. These were death (regardless of cause), nonfatal myocardial infarction (at least two of the following: typical pain, electrocardiographic changes suggesting acute myocardial infarction, cardiac enzymes more than twice the upper limit of normal), coronary artery bypass graft surgery (CABG), and repeat angioplasty at the same site. Events were classified as "procedural" (i.e., onset of event or decision to perform another procedure taken while the guide catheter was still in place), "early" (i.e., onset within 24 hours of guide catheter removal), or "late" (i.e., onset more than 24 hours after guide catheter removal). Another secondary end point was the presence and severity of angina pectoris as assessed by the Canadian Cardiovascular Society classification at last follow-up.

Statistical Methods and Analysis

The minimal sample size was estimated at the outset of the study to be 233 patients in each group on the assumption of a change of -0.40 ± 0.50 mm in mean minimal lumen diameter between postangioplasty and follow-up angiogram in the control group[5] and -0.25 ± 50 mm (i.e., a 30% difference) in the active drug group (two-sided test with an α error of 0.05 and a power of 0.90).

In the comparison between treatment groups for the primary angiographic end point, patients included were those who had a successful initial angioplasty, had a quantitatively analyzable PTCA angiogram, had follow-up angiogram made while on trial medication, and were compliant with trial medication (had used at least 80% of their trial medication during the intervening period and had not discontin-

TABLE 3. Clinical Baseline Data of 697 Patients Included in Analysis for Clinical End Points

	Control (n=346)		GR32191B (n=351)	
	n	%	n	%
Men (n)	276	80	279	80
Age (years)	56.9±9.0		56.6±9.8	
Ever smoked	259	75	280	80
Current smoker	40	12	57	16
Diabetes	28	8	29	8
History of hypertension	111	32	120	34
Lipids (mmol/l)				
Total cholesterol	6.2±1.1	(n=337)	6.2±1.2	(n=345)
LDL cholesterol	4.2±1.2	(n=240)	4.1±1.3	(n=251)
HDL cholesterol	1.1±0.5	(n=290)	1.2±0.5	(n=297)
CCS class				
I	37	11	42	12
II	111	32	116	33
III	141	41	140	40
IV	57	16	52	15
Pain at rest controlled by intravenous nitrates	43	12	48	14
Duration of angina (months)	2±44		24±45	
Previous MI	134	39	132	38
Previous CABG	7	2	12	3
Previous angioplasty	4	1	6	2
Patients on medication				
Nitrates	235	68	225	64
Calcium antagonists	208	60	222	63
β-Blockers	175	51	191	54
Monotherapy	97	28	104	30
Double therapy	151	44	156	44
Triple therapy	73	21	74	21

LDL, low density lipoprotein; HDL, high density lipoprotein; CCS, Canadian Cardiovascular Society angina classification; MI, myocardial infarction; CABG, coronary artery bypass graft surgery.

ued trial medication for more than 3 days). To test the null hypothesis that both mean changes in minimal lumen diameter are equal, an unpaired t test was used and a 95% confidence interval for the effect measure was obtained.

Comparisons for each clinical event were made on the basis of intention to treat (i.e., with inclusion of all patients who were randomized—defined as having taken at least their initial oral dose of trial medication—and regardless of angioplasty outcome or trial medication compliance). Also, the clinical status of each patient at the end of follow-up was ranked by assignment to the lowest applicable category of the following ordinal scale: 1, death; 2, nonfatal myocardial infarction; 3, status after CABG; 4, status after repeat PTCA; 5, presence of angina pectoris (Canadian Cardiovascular Society classification of 1 or higher); and 6, none of the above. The percentages of patients in each of these categories were compared between treatment groups on the basis of intention to treat. For all comparisons, the null hypothesis of no difference was tested by appropriate statistical tests.

Results

A total of 707 patients were randomized. Of these patients, 353 were randomized to receive GR32191B, and 354 were randomized to the control group. Selected demographic, clinical, and angiographic characteristics of the two study groups are shown in Tables 2 and 3. No baseline differences were observed between the two groups.

Figure 1 shows the patient flow and the reasons that subjects could not be evaluated with respect to quantitative angiographic restenosis. In 10 patients, angioplasty was not performed. One patient, who could not be treated because of radiographic equipment failure, was rerandomized 2 weeks later and retrospectively excluded as a protocol violator (previous participation in the trial was an exclusion criterion). Angioplasty was successful in 322 of the treated patients and 327 of the control group. Angioplasty was unsuccessful in 29 patients in the treated group and 19 in the control group. Thus, 322 treated patients and 327 control patients underwent successful angioplasty of at least one lesion and were eligible for follow-up angiography. Quantitative angiographic

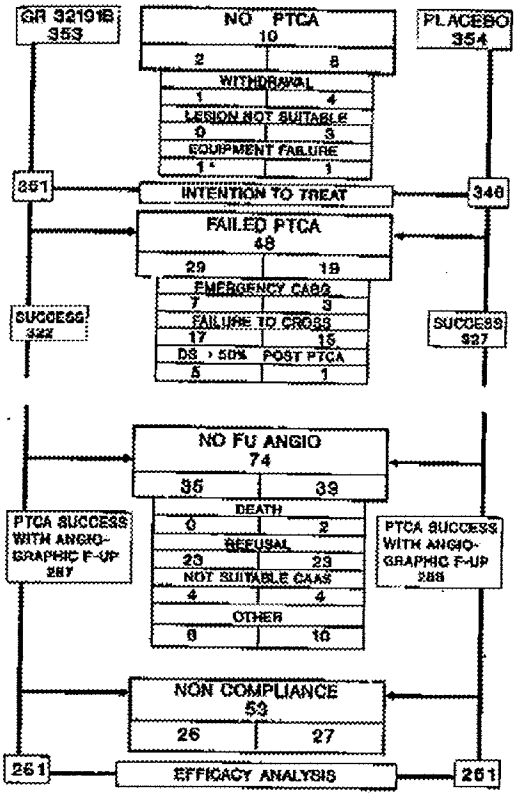

FIGURE 1. *Schematic of patient flow in Coronary Artery Restenosis Prevention on Repeated Thromboxane-Antagonism trial and reasons why no follow-up angiogram and/or quantitative angiography was obtained. Angio, coronary angiography; CABG, coronary artery bypass graft surgery; CAAS, coronary artery analysis system; DS, diameter stenosis; FU, follow-up; angioplasty, percutaneous transluminal coronary angioplasty. *Patient randomized twice and excluded from trial.*

follow-up as not available in 74 cases (35 treated and 39 control). In 18 cases, quantitative angiography could not be obtained for a variety of reasons: peripheral vascular problems ($n=3$), intercurrent noncardiovascular disease rendering repeat catheterization not desirable ($n=9$), one patient moved to another country, three patients underwent CABG without preoperative recatheterization, one cinefilm was lost, and one film was damaged during processing. Finally, 53 patients did not fulfill the compliance criteria and were excluded from the quantitative angiographic efficacy analysis (Figure 1).

Result of Angiographic Efficacy Analysis

Table 4 and Figure 2 summarize the quantitative angiographic findings of the efficacy analysis. At follow-up, the loss of minimal lumen diameter was identical in both groups: −0.31 mm (treatment effect, 0 mm; 95% confidence intervals, −0.09, 0.09). Figure 3 is a cumulative curve of the change in minimal lumen diameter observed in both groups. A loss of 0.72 mm or more[5,27] corresponds to restenosis rates of 19% in the control group and 21% in the treated group. Therefore, the relative risk for restenosis in the treated group with respect to the control group is 1.15 (95% confidence intervals, 0.82, 1.60).

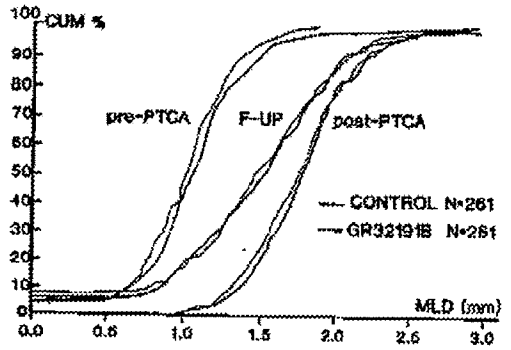

FIGURE 2. *Cumulative distribution curve of minimal lumen diameter before percutaneous transluminal coronary angioplasty (PTCA), post-PTCA, and at 6-month follow-up (F-UP) in both treatment groups. CUM %, cumulative percentage of patients; MLD, minimal luminal diameter.*

Results of Bicycle Ergometry

Of 649 patient who had a successful angioplasty, 539 underwent exercise testing at follow-up. Reasons for not performing the test were death (2 patients), unstable angina (45 patients), inability to perform the

TABLE 4. Quantitative Analysis of 636 Lesions in 522 Patients

	Control ($n=261$)	GR32191B ($n=261$)
Obstruction diameter (mm)		
Before angioplasty	0.99±0.35	1.06±0.39
After angioplasty	1.77±0.34	1.79±0.33
Follow-up	1.46±0.59	1.49±0.58
Reference diameter (mm)		
Before angioplasty	2.64±0.57	2.70±0.59
After angioplasty	2.71±0.54	2.76±0.48
Follow-up	2.72±0.55	2.74±0.52
Difference in obstruction diameter (mm)		
After angioplasty minus before angioplasty	0.78±0.39	0.73±0.38
Follow-up minus after angioplasty	−0.31±0.54	−0.31±0.55
Percentage stenosis (%)		
Before angioplasty	62±13	61±12
After angioplasty	34±9	34±9
Follow-up	46±19	45±19
Difference in percentage stenosis (%)		
After angioplasty minus before angioplasty	−28±14	−26±14
Follow-up minus after angioplasty	12±20	11±19

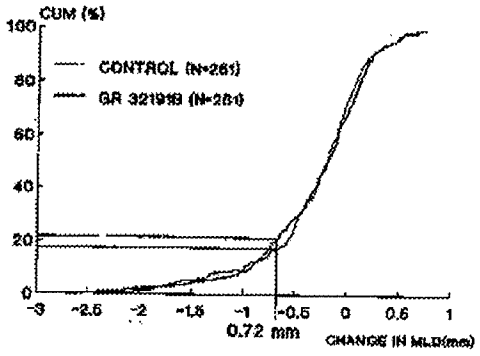

FIGURE 3. *Cumulative distribution curve of change in minimal lumen diameter from after angioplasty to follow-up in both treatment groups. CUM %, cumulative percentage of patients; MLD, minimal luminal diameter.*

test (19 patients), refusal (33 patients), and other (11 patients). Table 5 summarizes results of exercise testing in both groups. No difference in any parameter was observed at submaximal or maximal exercise. ST deviation (depression or elevation) of more than 0.1 mV (more than 1 mm) associated with anginal symptoms (considered positive) was observed in 47 patients in the control group and 55 patients in the GR32191B group.

Clinical Follow-up

Table 6 shows the total number of events during 6-month follow-up as well as the ranking of clinical status 6 months after angioplasty for all 697 patients randomized. Adjusted χ^2 test revealed no difference in ranking between the two groups. At 6-month follow-up, a comparable number of patients in both treatment groups were in each Canadian Cardiovascular Society class. Finally, 194 patients (56%) in the treated group and 197 (56%) in the control group were event and symptom free at 6-month follow-up.

TABLE 5. Exercise Test Results

	Control (n=262)		GR32191B (n=277)	
	n	%	n	%
Position				
Supine	98	38	110	40
Sitting	164	62	167	60
ST deviation >0.1 mV	102	39	117	42
Anginal symptoms during test	76	32	73	30
Combination of >0.1 mV segment deviation and symptoms	47	18	55	20
Maximum work load (W)	142±41		144±40	
Expected work load (W)	160±32		161±39	
Exercise time (minutes)	7.8±2.9		7.8±3.0	
Systolic blood pressure at peak exercise (mm Hg)	193±32		196±31	
Heart rate at peak exercise (min^{-1})	133±24		135±22	

Test performed in 539 of 649 patients with successful angioplasty.

Results of GR32191B Assay and Platelet Aggregation Tests

In four of the six participating centers, GR32191B plasma levels for patients allocated to the GR32191B group were analyzed before first drug intake and approximately 1 hour after first drug dose. GR32191B was not detected above the limit of quantification in the predose samples but was present in the postdose samples at concentrations ranging from 5 to 1,210 ng/ml, with a mean of 392±241 ng/ml, indicating that GR32191B was absorbed into the circulation after the oral administration of GR32191B.

During each follow-up visit of the Rotterdam patients, platelet aggregation tests were carried out using the TXA_2 mimetic U-46619 and ADP as aggregants. During the first three visits (3 weeks, 3 months, and 6 months after angioplasty), patients were on trial medication. The fourth visit (7 months after angioplasty) served as a control measurement. A total of 162 patients were tested at least one time during follow-up (Table 7). Mean ADP aggregation during visits 1, 2, and 3, expressed as peak response, was 116±12 mm (214 analyses) in the treatment group and 125±12 mm (203 analyses) in the control group (two-tailed t test, p=0.4). Mean U-46619 aggregation during visits 1, 2, and 3 was 10±21 mm (215 analyses) in the treatment group and 100±35 mm (203 analyses) in the control group (two-tailed t test, p<0.0001). This significant lowering of U-46619 aggregation in the treated group was observed in all except five patients during their 3-month test. These five patients showed U-46619 aggregation of more than 100 mm. At the 7-month assessment (patients off trial medication), mean U-46619 aggregation again rose to 80±42 mm (27 analyses) in the treatment group, which is not significantly different from the value of 99±32 mm (19 analyses) in the control group (two-tailed t test, p=0.8).

Bleeding Complications and Tolerability

Only mild bleeding events occurred in the trial. In-hospital bleeding events occurred in 18 patients (5%) in the control group and 15 patients (4%) in the treatment group (hematoma at puncture site of more than 5 cm, 14 versus 12 patients; prolonged bleeding at puncture site, three versus four; hematoma elsewhere, one versus none). During follow-up, four hematomas were reported in the control group and five in the treatment group. No cerebral bleeding or cerebral thrombotic events were encountered during the time course of the trial. Generally, the drug was well tolerated, and reported side effects were mild and evenly distributed in the two treatment groups. Total reported side effects were 40 in the control group and 44 in the treatment group (epigastric discomfort, 19 versus 20 patients; rash, 11 versus 12; nausea, six versus three; salivation, none versus two; headache, three versus six; fever, one versus one).

TABLE 6. Total Number of Events and Ranking Scale

	Total events during 6-month follow-up				Ranking of clinical status 6 months after angioplasty			
	Control (n=346)		GR32191B (n=351)		Control (n=346)		GR32191B (n=351)	
	n	%	n	%	n	%	n	%
Death								
Late	6		4					
All	6	2	4	1	6	2	4	1
Myocardial infarction								
Procedural	5		5					
Early	11		7					
Late	6		6					
All	22	6	18	5	22	6	18	5
Bypass graft surgery								
Procedural	3		7					
Early	5		2					
Late	18		18					
All	26	8	27	8	19	6	22	6
Repeat angioplasty								
Early	9		6					
Late	59		54					
All	68	20	60	17	52	15	49	14
CCS classification*								
IV	5	2	1	0.3	5	2	1	0.3
III	19	6	18	5	11	3	11	3
II	36	11	47	14	23	7	30	9
I	26	8	32	9	14	4	19	5
None	254	75	249	72	194	56	197	56

CCS, Canadian Cardiovascular Society angina classification. *For 687 patients alive at 6-month follow-up; secondary end point.

Discussion

Rationale for Selective Thromboxane Blockade in Prevention of Restenosis

At the time of the design of the trial in 1986, it was thought that platelet aggregation at the site of endothelial denudation and vascular disruption played a pivotal role in the pathogenesis of restenosis. Massive platelet deposition and aggregation at the dilatation site[13] could, on the one hand, lead to organization of a mural thrombus[28] and, on the other hand, trigger a fibroproliferative reaction of the vessel wall via the release of growth factors and chemotactic agents.

Balloon angioplasty causes a severe vascular trauma that can only be compared with spontaneous plaque rupture in unstable anginal syndromes,[29] with its known deleterious thrombotic consequences. Prevention of thrombotic events by blocking the TXA_2-induced aggregation with aspirin is known to be effective in unstable angina[30,31] and in the periangioplasty period.[32,33] Nevertheless, aspirin may still fail

TABLE 7. Platelet Aggregation Tests

Aggregation agent	Visit 1 (3 weeks)	Visit 2 (3 months)	Visit 3 (6 months)	Visit 4 (7 months)	Analysis of variance
ADP active (mm)	116±12	118±11	115±14	121±17	NS
n	81	72	61	26	
ADP control (mm)	124±13	126±11	127±11	118±11	$p=0.03$
n	81	69	53	19	
U46619 active (mm)	8±12	7±12	15±34	80±42	$p<0.0001$
n	81	72	62	27	
U46619 control (mm)	101±36	101±34	100±37	99±32	NS
n	81	69	53	19	

During visits 1, 2, and 3, patients were on trial medication.

short as the ideal agent because it is not sufficiently specific as an inhibitor of TXA_2 production.[34] Furthermore, by irreversibly acetylating cyclo-oxygenase and preventing the formation of the endoperoxide prostaglandin H_2, aspirin can block the production of "beneficial" prostaglandins such as prostacyclin as well as the "detrimental" TXA_2. A drug that preserved prostacyclin production while inhibiting the production or actions of TXA_2 might be expected to be superior to aspirin. This could be achieved either by a TXA_2 synthetase inhibitor or a TXA_2-receptor–blocking drug. However, no TXA_2 synthetase inhibitor is known to produce a complete blockage of TXA_2 synthesis. Furthermore, accumulating precursors of TXA_2, such as prostaglandin H_2, are also capable of inducing aggregation via the TXA_2-receptors.[35] In contrast, TXA_2-receptor blockade will antagonize not only the proaggregatory actions of TXA_2 but also those of agents that act indirectly via TXA_2, such as collagen, and agents that directly stimulate the TXA_2-receptor, such as prostaglandin H_2. A role for TXA_2-receptor blockade after PTCA has been suggested by an experimental animal model showing reduced intimal hyperplasia after balloon injury of rat carotids after treatment with GR32191 (M. Zimmerman, personal communication).

GR32191 (in doses of 0.125–1.0 mg/kg p.o) produced a dose-related antagonism of U-46619–induced platelet aggregation ex vivo, which at the 1-mg/kg dose persisted for more than 24 hours.[36] GR32191B has also been demonstrated to produce a long-lasting blockade of the TXA_2-receptor on vascular smooth muscle in vivo in humans.[36] Chronic dosing (17.5 mg b.i.d.) resulted in progressively increasing antagonism of U-46619–induced aggregation such that virtually complete inhibition was achieved over the entire 12-hour dosing cycle.[37] In healthy volunteers[37] as well as in our patients, the drug was well tolerated, and bleeding time was only slightly prolonged. Finally, GR32191 is entirely devoid of any agonistic actions.[38]

Trial Design

The design of CARPORT was based on four considerations, each of them having specific consequences. First, it was the underlying assumption that TXA_2-receptor blockade with GR32191B started before angioplasty would, at least in theory, affect both acute restenosis resulting from platelet aggregation–induced thrombus formation and chronic restenosis resulting from platelet aggregation–induced hyperplasia. Second, in view of the fact that patients in whom angioplasty did not succeed are not "at risk" for restenosis, trial medication was continued only in case PTCA was successful. Third, at this stage of the development of the therapeutic principle involved, it was considered necessary to establish the mechanism of action by direct observation of restenosis by angiography. As a consequence, the protocol included follow-up angiography regardless of clinical status. Within-patient change of minimal lumen diameter, as assessed by objective, quantitative measurements of coronary segments filmed in multiple matched projections, was chosen as primary end point. Furthermore, the number of patients was planned based on what was known about the reproducibility of this method rather than on the need to have sufficient power for detecting an effect on clinical outcome (which would have required a much larger number of patients). Fourth, it was considered unethical not to give any protection against acute thrombotic events during angioplasty to participating patients.[32,33] As a consequence, one dose of intravenous aspirin was given before PTCA to the control group before placebo was started.

Loss in Minimal Luminal Diameter as Primary End Point: A Noncategorical Approach

The reappearance of angina as a sole criterion of restenosis underestimates the angiographic rate of restenosis, and the value of recurrent anginal symptoms as a marker of restenosis is difficult to assess in many studies because the timing and completeness of angiographic follow-up often have been determined by symptomatic status.[39]

In the present trial, repeat catheterization with quantitative angiography was obtained in 88.5% of 649 patients with a successful angioplasty. A majority of patients (354 of 522, or 68%) were recatheterized in the 6-month (±2 weeks) time interval. The remaining patients underwent early recatheterization because of clinical suspicion for restenosis. Of the 522 compliant patients who had angiography at follow-up, 345 were angina free and 165 were symptomatic at follow-up. As shown in Figure 4B, there was considerable overlap between the change in minimal lumen diameter of patients with and of those without angina at follow-up angiography. This underscores that reappearance of angina is a poor proxy to the anatomic substrate at issue and confirms the poor predictive value of symptoms found in other studies,[29] which may be explained by the presence of other mechanisms for angina, such as incomplete revascularization or progression of disease in other vessels.

Several studies have examined the usefulness of ergometry to detect restenosis after angioplasty.[39] These studies have generally found that the presence of exercise-induced angina, ST segment depression, or both is not highly predictive of restenosis whether the test is performed early or late after angioplasty.[39] Figure 4B illustrates this for our data in a similar fashion as for angina. In view of the above, quantitative coronary angiography has emerged as the most reliable method for judging late results.

In studies evaluating the biology of restenosis, a continuous measure of the degree of lumen obstruction is preferable because any progression of the stenosis reflects the process of interest regardless of whether an arbitrarily defined threshold of obstruction is reached. Keeping in mind that an angiographic restenosis study assesses only the anatomic component of the restenosis problem, there is no

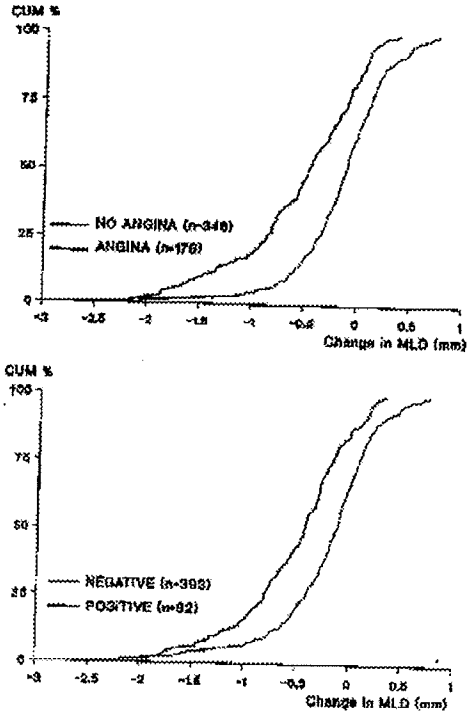

FIGURE 4. Top panel: *Cumulative distribution (CUM %) curve of change in minimal lumen diameter (MLD) for symptomatic and asymptomatic patients at follow-up angiography.* Bottom panel: *Cumulative distribution (CUM %) curve of change in minimal lumen diameter (MLD) for patients with positive ergometry and negative ergometry at follow-up angiography.*

threshold above which a loss of lumen diameter would have clinically significant functional or symptomatic consequences. Why, then, would one try to define a threshold above which there would be "significant" quantitatively determined angiographic restenosis? To define the threshold on consideration of reproducibility of the measurement in individual patients is also questionable. The expected benefit of a treatment can be measured with much greater precision by using the change in lumen diameter for the group. If it is assumed that treatment reduces the loss of lumen diameter from 0.4 mm under control conditions to 0.25 mm under active medication, 233 patients per treatment group are required for there to be a power of 90%. The above reduction corresponds with restenosis rates (defined as a loss of minimal lumen diameter of 0.72 mm or more) of 25% and 17.5%, respectively.[5,27] This difference, however, can be statistically detected only with 620 patients per treatment group (power, 90%). Thus, statistically, the quantitative outcome determined from direct measurements of continuous variables can be evaluated with only one third of the number of patients required for the categorical outcome. This is logical because categorical end points do not take full advantage of the available information.

Possible Explanations for Lack of Effect of GR32191B

In this trial, TXA_2-receptor blockade failed to demonstrate prevention of angiographic restenosis after angioplasty. Also, there was no apparent effect on overall clinical outcome. There are several possible explanations.

First, it could be hypothesized that the absence of benefit was due to poor absorption. In four participating clinics, plasma levels of GR32191B before first drug dose and 1 hour afterward confirmed an excellent gastrointestinal resorption of the drug in this group of patients with coronary artery disease who were fasting while awaiting an angioplasty procedure. Second, compliance could have been poor. Aggregation tests in one participating clinic showed that a 90% reduction of platelet aggregation via the TXA_2 pathway was achieved in the treated group throughout the entire study. This indicated that patients were taking their medication and that the drug was pharmacologically active. Third, it might be hypothesized that this substantial reduction in the aggregatory response of the platelet is still insufficient to prevent a substantial release of other factors involved in the initiation of the proliferative response.[13,40–42] In a recently published study, it was shown that GR32191 had no effect on primary aggregation induced by ADP, adrenaline, or platelet aggregating factor.[43] In the present study, GR32191 was found to inhibit only 70% of the total platelet deposition on deendothelialized rabbit aorta using ^{111}In-labeled human platelets from whole blood.[43] This was similar to the maximum inhibition achieved with prostacyclin and aspirin. Because several clinical trials with aspirin after balloon angioplasty have failed to prove a beneficial effect on restenosis,[32,44–46] it might be retrospectively inferred that a similar level of platelet inhibition would also fail to alter the restenosis rate. Furthermore, the magnitude of TXA_2-receptor blockade needed after balloon-induced vascular damage is not known. For example, a substantial increase in plasma levels of TXA_2 metabolite 11-dehydro-thromboxane B_2 from less than 50 to 174 pg/ml has been measured in the great cardiac vein after angioplasty of the left anterior descending coronary artery, despite pretreatment with aspirin.[47] One could question whether TXA_2-receptor blockade is effective in the face of such an increase, although it has been demonstrated that GR32191 can achieve a more-than-100-fold displacement to the right of the platelet aggregation concentration-effect curve for U-46619 in healthy subjects.[36]

More recently, it has been advocated that inhibition of platelet adhesion is a more efficient means to prevent subsequent aggregation of platelets.[48–51] However, it can be argued that complete inhibition of adhesion will cause untoward bleeding effects.

Finally, the pivotal role of the platelet in the initiation of the restenosis process might have been overestimated,[52] and antiplatelet therapy as the sole modality of treatment may be intrinsically insufficient to control the restenosis phenomenon.

Appendix

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KEY WORDS • percutaneous transluminal coronary angioplasty • restenosis • quantitative angiography • clinical trials

EDITORIAL

Rapamycin eluting stent: the onset of a new era in interventional cardiology

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Drug eluting stents represent one of the fastest growing fields in interventional cardiology today.

At the congress of the European Society of Cardiology in Amsterdam in 2000, I (PWS) was asked to give the Andreas Gruentzig Lecture. In the week preceding the lecture, we re-angiographied patients 32 and 33 of the initial cohort of patients who had received a rapamycin eluting stent in Sao Paulo and in Rotterdam. Scrutinising the 4–6 month angiographic and ultrasonic results of these patients, I became overwhelmingly convinced that we were the privileged witnesses of a new phenomenon: the almost complete abolition of intra-stent neointimal proliferation. Colleagues, invasive and non-invasive cardiologists, old friends, and financial analysts were surprised by the unusual "excess of enthusiasm" coming from somebody who has built over the years a reputation as a critical assessor, never one to be carried away by the hype of a new wave in interventional cardiology. In the history of this field I have recognised (and "got excited" by, as my American colleagues used to put it) only two revolutionary developments: the introduction of the moveable and steerable guidewire by John Simpson, and the advent of the stent (Palmaz-Schatz, Wallstent). The drug eluting stent is the third such development, and almost one year later I would like to restate the fact that we are entering a new era in interventional cardiology. Why? Because the principle of an eluting stent is sound, and because the three major technical challenges have been mastered—the controlled release of an efficient drug from a stable coating.

THE PRINCIPLE

Drug administration for the prevention of restenosis has been tested in the past—with disappointing results throughout. A proposed explanation for the repeated failure of clinical drug studies has been that agents given systematically cannot reach sufficient concentrations in injured arteries, which has a signficant impact on the restenotic process. Local drug administration offers advantages. The active drug is applied to the vessel at the precise site and at the time of vessel injury. Local drug delivery is able to achieve higher tissue concentrations of the drug. No additional material or procedures are required. Systemic release is minimal and may reduce the risk of remote systemic toxicity.

See end of article for authors' affiliations

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THE DELIVERY VEHICLE

The delivery vehicle must fulfil pharmacological, pharmacokinetic, and mechanical requirements. The release of the drug into the vessel must take place in a manner that is consistent with the drug's mode of action. Drug release must be predictable and in a controlled concentration and time. The delivery vehicle must be suitable for sterilisation; it must follow the geometric change of configuration during stent expansion and resist mechanical injury caused by the inflation of the balloon. Today these problems are controlled, guaranteeing intact coating during clinical application.

THE DRUG

The drug should be one that inhibits the multiple components of the complex restenosis process. Uncontrolled neointimal tissue accumulation shows some parallels to tumour growth, thus the use of antitumorous strategies seems to be a logical consequence. Numerous pharmacological agents with antiproliferative properties have been tested for their potential to inhibit restenosis.

Rapamycin (sirolimus) has been approved by the US Food and Drug Administration for the prophylaxis of renal transplant rejection. It is a naturally occurring macrocyclic lactone which is highly effective in preventing the onset and severity of disease in several animal models of autoimmune disease, such as insulin dependent diabetes mellitus, systemic lupus erythematosus, and arthritis.

RAPAMYCIN'S MECHANISM OF ACTION

The class of macrocyclic immunosuppressive agents (rapamycin, cyclosporin A, tacrolimus FK506) bind to specific cytosolic proteins called immunophilins (for example, FK506 binding protein 12) to gain their immunosuppressive activity. Rapamycin blocks G1 to S cell cycle progression by interacting with a specific target protein (mTOR, mammalian target of rapamycin) and inhibits its activation. The inhibition of mTOR suppresses cytokine driven (IL-2, IL-4, IL-7, and IL-15) T cell proliferation. mTOR is a key

Abbreviations: FR, fast release; IL, interleukin; IVUS, intravascular ultrasound; mTOR, mammalian target of rapamycin; PBMA, polybutylmethacrylate; PCNA, proliferating cell nuclear antigen; PEVA, polyethylenevinylacetate; RAVEL, randomised study with sirolimus coated BX Velocity balloon expandable stent in the treatment of patients with de novo native coronary lesions; SR, slow release; VEGF, vascular endothelial growth factor

regulatory kinase and its inhibition has several important effects, including: the inhibition of translation of a family of mRNAs that code for proteins essential for cell cycle progression; the inhibition of IL-2 induced transcription of proliferating cell nuclear antigen (PCNA) that is essential for DNA replication; the blocking of CD28-mediated sustained upregulation of IL-2 transcription in T cells; and the inhibition of the kinase activity of the cdk4/cyclinD and cdk2/cyclinE complexes, essential for cell cycle progression. The mechanism of action is distinct from other immunosuppressive drugs that act solely by inhibiting DNA synthesis, such as mycophenolate mofetil and azathioprine. Rapamycin is synergistic with cyclosporin A and has much lower toxicity than other immunosuppressive agents.

Rapamycin prevents proliferation of T cells but also proliferation[1] and migration of smooth muscle cells. Gregory and colleagues demonstrated that intraperitoneal administration of rapamycin resulted in a dose dependent inhibition of arterial intimal thickening caused by either chronic alloimmune or mechanical injury in a rat model.[2 3] Subsequent studies reported that rapamycin inhibited both human and rat vascular smooth muscle cell proliferation in vitro by blocking G_1/S transition. The inhibition of proliferation was mediated by rapamycin binding to its cytosolic receptor, FK506 binding protein 12, and associated with reduced cdk2 activity and protein retinoblastom phosphorylation.[4 5]

Gallo and colleagues recently showed that systemic rapamycin treatment significantly reduces the proliferative response after coronary angioplasty in the porcine model.[6] The antiproliferative effects of rapamycin after angioplasty were attributed to an inhibition of the pRB phosphorylation preventing the down regulation of $p27^{kipt}$. Thus, the antiproliferative activity of rapamycin after balloon arterial injury in conjunction with its immunosuppressive properties suggests that this drug could also be useful for the prevention of in-stent restenosis.

This hypothesis is further supported by findings in human carotid arteries.[7] A robust upregulation of FK506 binding protein 12 was detected in the neointimal tissue of restenotic lesions, whereas no FK506 binding protein 12 was detectable in smooth muscle cells from control media.

THE RAPAMYCIN ELUTING STENT

The rapamycin coated BX Velocity stent is fabricated from medical 316 LS stainless steel. It is available in a length of 18 mm and in two cell configurations (6 cell configuration: expanded diameter 2.5–3.25 mm) and 7 cell design (expanded diameter 3.5–3.75 mm). The stent contains 140 μg rapamycin/cm^2 which gives a total rapamycin content of 153 μg on the 6 cell stent and 180 μg on the 7 cell stent. The coating formulation consists of 30% rapamycin by weight in a 50:50 mixture of the polymers polyethylenevinylacetate (PEVA) and polybutylmethacrylate (PBMA).

IN VIVO PHARMACOKINETICS

In vivo pharmacokinetics studies in the porcine coronary model demonstrated that the whole blood concentration of rapamycin peaks at 1 hour (mean (SD) 2.63 (0.74) ng/ml) after stent deployment and then declines below the lower limit of detection (0.4 ng/ml) by three days. The total arterial tissue concentration of rapamycin is 97 (13) ng/artery and the residual stent content is 71 (10) μg at three days. The amount of residual rapamycin on the stent at three days is 43% of the initial quantity loaded on the stent. A modification of the coating provides similar arterial tissue concentrations at 28 days. These data document the ability to deliver and achieve a potentially therapeutic arterial tissue concentration of rapamycin in the porcine model and insignificant concentrations in the systemic circulation using the non-erodible methacrylate and ethylene based copolymer matrix.

PRECLINICAL EFFICACY STUDIES

Preclinical efficacy studies demonstrated a 35–50% reduction in in-stent neointimal hyperplasia for the rapamycin coated stents as compared with bare metal stents at 28 days in the porcine and rabbit model.[8] Histological assessment revealed the presence of typical cellular components of the neointima and a similar degree of re-endothelialisation for the rapamycin as compared with the bare metal stents. The morphology of non-stented reference arterial wall sections, including the vessel area, neointimal area, and per cent area stenosis was similar for the metal and each of the drug coated stents. A semiquantitative histological grading system demonstrated less smooth muscle cell colonisation and more residual fibrin deposition for the rapamycin eluting stents as compared with the bare metal stents. Therefore, critical reparative events, such as endothelialisation and smooth muscle cell colonisation of the neointima, with rapamycin eluting stents occur in a similar temporal sequence as observed with bare metal stents. The focal remnants of residual fibrin deposition observed in the vessel with rapamycin coated stents may reflect a delay in arterial repair or impaired fibrin degradation secondary to the local effects of the drug.

CLINICAL DATA

The first clinical application of the rapamycin coated stent was performed in Sao Paulo and Rotterdam. Thirty patients with angina pectoris were electively treated with two different formulations of the rapamycin coated BX Velocity stent (Cordis) (slow release [SR] n = 15, and fast release [FR], n = 15). All stents were successfully delivered, and patients were discharged without clinical complications. At four months' follow up, there was minimal neointimal hyperplasia in both groups as assessed by IVUS and quantitative coronary angiography (in-stent late loss, 0.09 (0.03) mm [SR] and 0.02 (0.3) mm [FR]). No in-stent or edge restenosis was observed. No major clinical events (stent thrombosis, repeat revascularisation, myocardial infarction, death) had occurred by 12 months.[9] At one year follow up, IVUS volumetric analysis and angiography indicated minimal amounts of neointimal hyperplasia that were scarcely different from the four month data in both groups, with some patients showing no evidence of hyperplasia whatsoever. There were no major adverse cardiac events and no restenosis in either of the groups. One late acute myocardial infarction occurred in the FR group at 14 months.[10] In Rotterdam,15 patients were treated, and quantitative angiography and three dimensional quantitative IVUS were performed at implantation and at six months' follow up. All stent implantations were successful; one patient died on day 2 of cerebral haemorrhage and one patient suffered subacute stent occlusion caused by edge dissection. At nine months' follow up no further adverse events had occurred and all patients were angina-free. Quantitative coronary angiography revealed essentially no change in minimal lumen diameter and per cent diameter stenosis by angiographic criteria, and hence no in-lesion or in-stent angiographic restenosis was observed. Quantitative ultrasound showed that intimal hyperplasia volume and per cent obstruction volume at follow up were negligible (5.3 mm^3 and 1.8%, respectively). No edge effect was observed in the segment proximal and distal to the stent.[11]

These first clinical results are spectacular, as they convincingly demonstrate the absence of neointimal proliferation in all patients within the first six months after coronary stent implantation, a phenomenon which has never been reported in the past. If this promise—namely, the elimination of restenosis—becomes reality we will witness the onset of a new era in interventional cardiology and the revolution of catheter based intervention, bypass surgery, and health care economics! These enormous potential implications are the key for

today's enthusiasm. However, more than 20 years of experience in the investigation of restenosis force us to think of a possible Achilles' heel. In fact, a lot of unanswered questions still have to be resolved. First of all, controlled clinical data are needed. Furthermore, long term studies are required to elucidate if the drug is permanently inhibiting neointima growth or simply delaying the formation of neointima. Additionally, the recent experience with vascular brachytherapy alerts us to search for "unexpected" phenomena such as positive remodelling, late stent malapposition, edge effect, or late thrombosis. Again, meticulous long term clinical, angiographic, and IVUS follow up will be mandatory.

ONE YEAR LATER: DOES THE RAVEL STUDY REVEAL THE FULL STORY?

The randomised study RAVEL, using the rapamycin coated BX Velocity balloon expandable stent in the treatment of patients with de novo lesions in native coronary arteries, is a multicentre, prospective, randomised double blind clinical trial comparing a bare metal stent with the drug coated stent. Two hundred and twenty patients were randomised to a single rapamycin coated stent (140 $\mu g/cm^2$) versus a bare metal BX Velocity stent. At six months' follow up, the restenosis rate of the treated group was zero, the loss in minimal lumen diameter was zero, there was no target lesion reintervention, and the event-free survival was 96.5%.[12]

UPCOMING CLINICAL TRIALS

The SIRIUS study is a multicentre, prospective, randomised double blind trial that is being conducted in 55 centres in the USA. Eleven hundred patients with focal de novo native coronary arterial lesions (2.5–3.5 mm diameter, 15–30 mm long) will be randomised to treatment with rapamycin coated or bare metal BX Velocity balloon expandable stents. The primary end points of the SIRIUS trial are target vessel failure (death, myocardial infarction, target lesion revascularisation) at nine months. In addition, secondary end points are core laboratory analysis of angiographic and IVUS data to determine treatment effects on neointimal hyperplasia and in-stent restenosis. Clinical follow up will extend to three years in order to assess for late events. In addition to the pivotal RAVEL and SIRIUS trials, feasibility studies are ongoing to assess efficacy of rapamycin coated stents in more complex lesion subsets such as in-stent restenosis.

Drug eluting stents represent one of the fastest growing fields in interventional cardiology today. The exploitation of different classes of drugs which are potential candidates for the inhibition of restenosis, in combination with novel drug delivery systems or local gene therapy (for example, local expression of proliferation regulatory genes, transfer of cytotoxic genes, vascular endothelial growth factor (VEGF)) will continue. The multicentre trials will help to answer some of the most important clinical questions and determine whether this really reflects the eve of a "new era" or just a "new vogue" in interventional cardiology.

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Inter Partes Reexamination No. 95/001,096
Declaration of Campbell Rogers, M.D.
Exhibit 96

A randomized comparison of a durable polymer Everolimus-eluting stent with a bare metal coronary stent: The SPIRIT first trial

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Conflict of interest: All authors declare no conflict of interest.

Abstract

Background: Everolimus is a sirolimus analogue with similar efficacy in animal models, and has been previously successfully tested in humans using an erodable polymer.

Methods: This first-in-man single blind multi-centre randomized controlled trial assessed the safety and efficacy of everolimus eluting from a durable polymer on a cobalt chromium stent in patients with *de novo* native coronary artery lesions. Sixty patients were allocated to stent implantation with an everolimus-eluting stent (n=28) or an identical bare stent (n=32). Patients had either stable, unstable angina or silent ischaemia. Suitable lesions treated were single *de novo* native coronary lesions with 50-99% stenosis and could be covered by a 18 mm stent. The primary endpoint was in-stent late loss at 180 days, analysed on a per treatment basis. The major secondary endpoint was percent in-stent volume obstruction (%VO) as measured by intravascular ultrasound (IVUS) at 180 days. The clinical secondary endpoint was major adverse cardiac events (MACE) at 180 days.

Results: At 6 months, (matched pairs angiographic analysis), the in-stent late loss, percentage diameter stenosis and percentage of patients with binary restenosis were 0.10 mm, 16% and 0% respectively, in the everolimus arm (n=23), as compared with 0.87 mm, 39% and 25.9%, respectively in the bare stent arm (n=27, p<0.001 for late loss and diameter stenosis, p = 0.01 for restenosis). Significantly less neointimal hyperplasia was observed in the everolimus group compared to the bare stent group (10 ± 13 mm^3 vs 38 ± 19 mm^3, p<0.001) and similarly, less volume obstruction (8.0 ± 10.4% versus 28.1 ± 14.0%, p<0.001). A major adverse cardiac event occurred in 2 patients in the everolimus arm versus 6 in the bare stent arm.

Conclusion: Everolimus eluted from a durable polymer on a cobalt chromium stent effectively suppresses neointimal growth at 6 months compared to an identical bare stent.

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Introduction

Recent studies that have evaluated the local application of anti-proliferative drugs (sirolimus and paclitaxel) for the prevention of restenosis via a stent delivery system have shown that these therapies successfully inhibit the development of neointimal hyperplasia[1,2].

Everolimus is an effective anti-proliferative agent[3]. On a molecular level, everolimus forms a complex with the cytoplasmic protein FKBP12. In the presence of everolimus, the growth factor-stimulated phosphorylation of p70 S6 kinase and 4E-BP1 is inhibited. The latter proteins are key proteins involved in the initiation of protein synthesis. Since phosphorylation of both p70 S6 kinase and 4E-BP1 is under the control of mammalian Target Of Rapamycin (mTOR), this finding suggests that, like sirolimus, the everolimus-FKBP12 complex binds to and thus interferes with its function. Disabling mTOR explains the cell cycle arrest at the late G1 stage caused by everolimus and sirolimus.

The feasibility of using everolimus on a drug eluting stent was determined by the FUTURE I trial[4]. This trial utilized an S-stent and bioabsorbable polymer system (both Biosensors International, Singapore) and confirmed the safety of the everolimus-eluting stent at 6 and 12 months. At 6 months, a 7.7% Major Adverse Cardiac Event (MACE) rate was observed with no thrombosis and no late incomplete apposition. The efficacy was demonstrated by significant reduction of in-stent tissue proliferation at 6 months: both angiographic in-stent late loss and IVUS% neointimal volume were reduced by 87%. No angiographic in-stent binary restenosis was observed in the everolimus-eluting stent arm. The 12 month FUTURE I results showed sustained safety and efficacy with no new MACE events, no aneurysms, no late stent malapposition, and no thrombosis observed between 6 and 12 months. Minimal Lumen Area and Luminal Volume Index were maintained up to 12 months and no in-stent binary restenosis was observed up to 12 months.

The SPIRIT First clinical trial represents the first clinical evaluation of the Guidant XIENCE™ V Everolimus Eluting Coronary Stent System (XIENCE™ V Everolimus Eluting CSS), to investigate the potential benefits of the local application of everolimus in a durable polymer in combination with a thin strut cobalt chromium stent.

Methods

Patient selection

This randomized single-blind trial was performed at 9 medical centers and enrolled patients from December 2003 to April 2004. It was approved by the ethics committee at each participating institution, and all patients gave written informed consent.

Patients were eligible for the study if they were aged above 18 years and had received a diagnosis of stable or unstable angina or silent ischaemia. Additional eligibility criteria were the presence of a single primary *de novo* coronary lesion that was 3.0 mm in diameter as assessed by on-line QCA, that could be covered by an 18 mm stent, a stenosis of between 50-99% of the luminal diameter, and a Thrombolysis In Myocardial Infarction (TIMI) flow grade of 1 or more. Patients were not eligible for enrollment if they had an evolving myocardial infarction, stenosis of an unprotected left main coronary artery, an ostial location, located within 2 mm of a bifurcation, a lesion with moderate to heavy calcification, an angiographically visible thrombus within the target lesion, a left ventricular ejection fraction of less than 30%, were awaiting a heart transplant, or had a known hypersensitivity or contraindication to aspirin, heparin, clopidogrel, cobalt, chromium, nickel, tungsten, everolimus, acrylic and fluoro polymers or contrast sensitivity that could not be adequately pre-medicated.

The Everolimus-eluting stent

The Guidant XIENCE™ V Everolimus Eluting CSS is comprised of the Guidant MULTI-LINK VISION® Stent and delivery system, and a drug eluting coating. The Guidant MULTI-LINK VISION® Stent is a balloon expandable stent, which consists of serpentine rings connected by links fabricated from a single piece of medical grade L-605 cobalt chromium alloy.

Everolimus is blended in a nonerodable polymer (this drug layer was coated over another nonerodable polymer primer layer). This coating includes of acrylic and fluoro polymers, both approved for use in blood contacting applications. This layer of everolimus-polymer matrix with a thickness of 5-6 microns is applied to the surface of the stent and is loaded with 100 micrograms of everolimus per square centimeter of stent surface area with no top coat polymer layer. The stent is designed to release approximately 70% of the drug within 30 days after implantation.

Everolimus (Certican®, Novartis Corporation) has been evaluated in clinical trials in the US and Europe for use as an immunosuppressant following cardiac and renal transplantation[5]. Everolimus has received market approval in the European Union.

Study procedure

Following the confirmation of angiographic inclusion and exclusion criteria and prior to the procedure, patients were allocated through a telephone randomization service and assigned in a 1:1 ratio to either an everolimus eluting stent or bare metal stent. A single stent 3.0 mm in diameter, 18 mm long was used in the study.

Lesions were treated using standard interventional techniques with mandatory pre-dilatation and stent implantation at a pressure not exceeding the rated burst pressure. Due to packaging differences, physicians were not blinded to the device. Post-dilatation was allowed with a balloon shorter than the implanted stent. In the event of a dissection occurring at the edge of the implanted stent, it was recommended that a single additional bare Guidant MULTI-LINK VISION® stent be implanted as animal data only on single everolimus stent implantation were available at the onset of the study; these patients were *a priori* excluded from the per-treatment analysis but are part of the acute success population. IVUS was performed after angiographically optimal stent placement had been obtained and was repeated if additional post-dilatation was performed.

Intravenous boluses of heparin were administered according to local standard practice. Treatment with aspirin, at a minimum dose of 80 mg per day, was started at least 24 hours before the procedure and continued indefinitely. A loading dose of 300 mg of clopidogrel was administered 24 hours before the procedure, followed

by 75 mg daily for three months. Treatment with ticlopidine was permitted in case of clopidogrel hypersensitivity. Device success was defined as a final in-stent diameter stenosis of less than 50 percent by QCA using the assigned device. Clinical success was defined as the successful implantation of any device, with stenosis of less than 50 percent of the vessel diameter by QCA and no major cardiac events during the hospital stay.

Follow-up

Patients were evaluated at 30 days and 6 months. Further evaluations will be performed at 9 months and 1 year, with annual evaluations out to 5 years. At outpatient visits, patients were asked specific questions about the interim development of angina according to the Canadian Cardiovascular Society classification of stable angina. They were also monitored for MACE. Angiographic and IVUS evaluations were performed at 6 months, and will be repeated at 1 year. Prior to performing a follow-up angiogram, the physician was required to record in the source documents whether a revascularization (if required) was clinically indicated – defined as the presence of ischaemic symptoms and/or a positive functional ischaemia study.

Quantitative coronary angiography evaluation

Quantitative coronary angiography was performed using the CAAS II analysis system (Pie Medical BV, Maastricht, Netherlands). In each patient, the stented segment and the peri-stent segments (defined by a length of 5 mm proximal and distal to the stent edge) were analyzed. The following QCA parameters were computed: computer-defined Minimal Luminal Diameter (MLD), reference diameter obtained by an interpolated method, and percentage diameter stenosis. Binary restenosis was defined in every segment as diameter stenosis ≥50% at follow-up. Late loss was defined as the difference between MLD post-procedure and MLD at follow-up. Results are presented as matched pairs in the manuscript and as unmatched pairs in the Appendix. Unmatched pairs data is most commonly presented and utilises the mean QCA results of all projections obtained. Matched pairs data is more accurate as it compares the same views post-procedure and at follow-up and uses only QCA data of identical projections.

Intravascular ultrasound analysis

Post-procedure and follow-up stented vessel segments were examined with mechanical or phased array intravascular ultrasound using automated pullback at 0.5 mm per second. The coronary segment beginning 5 mm distal to and extending 5 mm proximal to the stented segment was examined. A computer-based contour detection program was used for automated 3-D reconstruction of the stented and adjacent segments. The lumen, stent boundaries and external elastic membrane (vessel boundaries) were detected using a minimum cost algorithm. The Stent Volume (SV) and Lumen Volume (LV) were calculated according to Simpson's rule. The intra-stent neointimal volume was calculated as the difference between SV and LV. The percentage obstruction of the stent volume was calculated as intra-stent neointimal volume/stent volume*100. Feasibility, reproducibility and inter- and intra-observer variability of this system have been validated *in vitro* and *in vivo*[6]. Incomplete apposition was defined as one or more stent struts separated from the vessel wall with evidence of blood speckles behind the strut on ultrasound, while late incomplete apposition was defined as incomplete apposition of the stent at follow-up which was not present post-procedure.

Study endpoints

The primary angiographic endpoint was in-stent luminal late loss, as determined by quantitative angiography. Secondary endpoints (QCA and IVUS) at 6 months and 1 year included the in-stent and in-segment late loss, angiographic binary restenosis rate, percentage diameter stenosis; and in-stent percentage volume obstruction. In-stent was defined as within the margins of the stent while in-segment was defined as located either within the margins of the stent or 5 mm proximal or distal to the stent. Late loss was calculated as the difference between the follow-up and post-procedure minimum luminal diameter. Secondary clinical endpoints were a composite of major cardiac events, including cardiac death, Q-wave or non-Q-wave myocardial infarction, clinically driven surgical or percutaneous revascularization of the target lesion (MACE) or vessel (Target Vessel Failure) at 30 days, 6 months, 9 months, and annually up to 5 years after the index procedure; and acute device, procedure and clinical success. All deaths that could not be clearly attributed to another cause were considered cardiac deaths. A non-Q-wave myocardial infarction was defined by an increase in the creatine kinase level to more than twice the upper limit of the normal range, accompanied by an increased level of creatine kinase-MB, in the absence of new Q waves on electrocardiography.

The endpoints were adjudicated by an independent clinical events committee. In addition, a data and safety monitoring board that was not affiliated with the study sponsor reviewed the data to identify any safety issues related to the conduct of the study.

Statistical analysis

The primary endpoint and all trial endpoints were analyzed on the per-treatment evaluable population which consisted of patients who had no bailout stenting and no major protocol deviations, as evaluated in a blinded manner. Acute success was analyzed on the entire patient population.

The sample size for the study was determined based on the primary endpoint of in-stent late loss at 180 days and on the following assumptions: a single comparison of active to uncoated; one-tailed t-test, unequal and unknown variances in the two groups being compared; $_{\alpha}$=0.05; true mean difference between the bare stent group and the treatment group of 0.48 mm. This assumption was made based on the results of the VISION Registry (mean late loss=0.83 mm)[7], SIRIUS trial (mean late loss=0.17 mm)[8] and TAXUS IV trial (mean late loss=0.39 mm)[9]. (Assume the true mean late loss for the treatment group is 0.35 mm, the difference between the bare stent group and treatment group is calculated as: 0.83 mm - 0.35 mm = 0.48 mm). The standard deviation was assumed to be 0.56 mm in the bare stent group and 0.38 mm in the treatment group (based on the results of the VISION Registry study and SIRIUS trial); approximately 20% rate of lost to follow-up or dropout; approximate-